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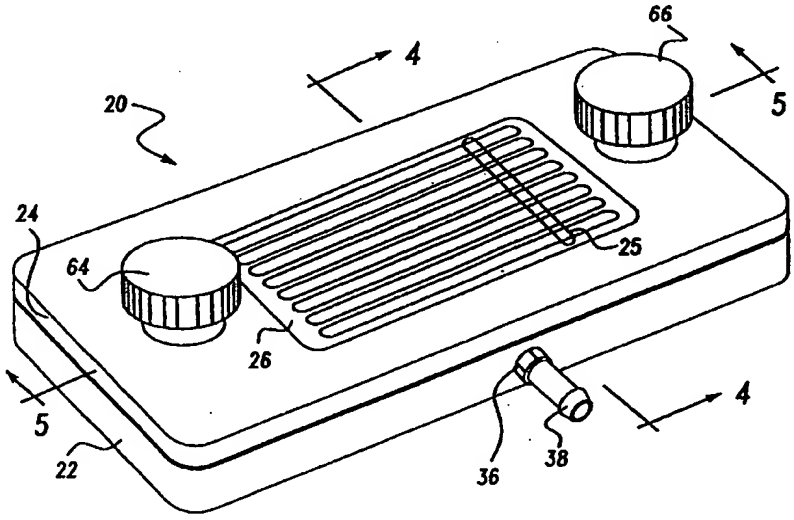

(57) Abstract

The invention includes assay cassettes that can be employed during rapid flow-through binding assays. The assay cassettes can be disposable units suitable for one-time use and readily assembled to include a filter membrane carried between an upper plate and a lower plate. A pattern of channels can extend through the top plate to allow a fluid sample to be applied through the top plate and onto the filter. The bottom plate can include a plurality of channels that are aligned with the channels of the top plate and which will allow a negative pressure to be applied to the underside of the filter membrane to draw the sample through the filter. In one embodiment, the cassette includes a frangible section that allows the cassette to be divided into a first and second component.

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RAPID FLOW-THROUGH BINDING ASSAY APPARATUS AND METHOD

FIELD OF THE INVENTION

The invention relates generally to improved devices for performing binding assays and, more particularly, to rapid flow-through binding assay apparatus and methods.

BACKGROUND OF THE INVENTION

Binding assays are routinely used to screen for and diagnose a host of diseases and conditions, including Lyme disease, herpes, acquired immunodeficiency syndrome (AIDS), streptococcal infections, lupus and pregnancy. Such assays are relatively simple in theory, utilizing the binding affinity between two or more binding members to detect and/or quantify the presence of one of the members, referred to herein as the analyte. Binding members comprise a wide range of substances, including antigens, antibodies, haptens, complimentary nucleic acid sequences, ligands, small molecules and receptors. Antigen-antibody binding member pairs used in immunoassays currently enjoy the most widespread use.

A common format of a binding assay involves immobilizing a binding member specific for the analyte on a paper-like sheet or membrane. The membrane is then contacted with the test sample and appropriate reagents under conditions allowing binding to occur between the immobilized binding member and any analyte in the sample, with means for detecting binding events also provided. Often a labeled second binding member which binds to the first binding member-analyte complex is added to provide a detectable signal on the membrane.

The sandwich immunoassay is an example of one commonly used binding assay for antibody detection. In a generic sandwich immunoassay, the antigen is immobilized on a solid substrate. Antibody containing solution, *e.g.*, diluted serum, is incubated with the immobilized antigen. Antibodies specific to the antigen bind to it, and unbound antibodies are then

removed by buffer washes. A detection agent which may typically be a secondary antibody conjugated to an enzyme, is then incubated with the primary antibody-antigen complex. Finally, an enzyme substrate is added which is converted into a visual, detectable product whenever the enzyme is . Such multi-step sandwich immunoassays can be developed in many different ways depending on assay requirements.

A Western Blot is another example of a commonly used immunoassay. In current practice, the Western Blot method comprises a sequence of incubation and wash steps performed on a membrane bearing electrophoretically resolved antigen bands. Typically, the membrane is cut into narrow strips, each bearing the identical pattern of antigen bands. Strips are then processed in reagent solutions individually in narrow trays, each typically holding 0.5-2.0 ml. In the first step, the strip is incubated with a blocking solution containing a non-specific protein, *e.g.*, non-fat dry milk, bovine serum albumin, newborn calf serum or gelatin. After washing off excess blocking solution with a wash buffer, typically a physiological saline buffer containing a low percentage of detergent, the strip is then incubated with antibody solution. Antibody solution may be diluted human or animal serum, cerebrospinal fluid, dried blood spot eluate, monoclonal antibody, to name a few. Unbound antibody is then washed off with buffer, and the strip is incubated in the detection reagent. In a typical application, the detection reagent could be goat-anti-human IgG-alkaline phosphatase conjugate. Unbound detection reagent is washed off with buffer, and finally the substrate (for alkaline phosphatase, a common substrate is 5-bromo-4-chloro-3-indolyl phosphate plus nitroblue tetrazolium) for the detection enzyme is added. The conversion of the substrate to a visually detectable product is allowed to proceed until optimal visualization of bands, and then substrate is washed away. The strip is typically dried, providing a permanent record of the assay result. Bands on the strip indicating antibody reactivity can be compared with control strips to determine the specificity of the immunoreaction. In currently used algorithms for HIV and Lyme testing, a positive test result is defined as the appearance of certain combinations of specific bands. For example, an HIV Western Blot test requires the presence of two bands to be considered positive, while a Lyme Western Blot test requires five out of ten bands to be positive for IgG, or two out of three bands to be positive for IgM.

As described above, the Western Blot method involves incubating the membrane strips sequentially in reagent solutions usually contained in a tray. In typical protocols, incubations with antibody solutions and detection reagents may take 30 minutes to several hours each. Wash steps may take 5 - 10 minutes each. The total time for processing a blot is therefore, not less than one hour, and is often several hours.

It would thus be desirable to provide a method and apparatus for the rapid processing of a binding assay, *e.g.*, an immunoassay, including a Western Blot.

SUMMARY OF THE INVENTION

The invention includes, *inter alia*, systems and methods for providing assay cassettes that can be employed during rapid flow-through binding assays. The assay cassettes can be disposable units suitable for one-time use and readily assembled to include a filter membrane carried between an upper plate and a lower plate. A pattern of channels can extend through the top plate to allow a fluid sample to be applied through the top plate and onto the filter. The bottom plate can include a plurality of channels that are aligned with the channel of the top plate and which will allow a negative pressure to be applied to the underside of the filter membrane to draw the sample through the filter. In one embodiment, the cassette includes a frangible section that allows the cassette to be divided into at least a first and second component.

More specifically, in one embodiment, the cassettes comprise a multi-channeled top plate that can be received into a multi-channeled bottom plate to define an interior chamber between the top plate and the bottom plate. A membrane having antibodies bound thereon can be received within the interior chamber and sandwiched between the top plate and bottom plate. To this end, the bottom plate and top plate can include an engagement mechanism, such as a notch and catch assembly that allows the top plate and bottom plate to be joined together. The bottom plate and top plate can be joined together in such a manner that a membrane placed into the interior chamber of the cassette is sandwiched between the bottom surface of the top plate and the top surface of the bottom plate. The assembled cassette with the membrane therein can be placed in an assay machine, such as a machine of the type manufactured and sold by the Immunetics Company of Cambridge, Massachusetts, USA. In these machines the cassette can be compressed

by the plates of a container that can compress together the top plate and bottom plate of the assay cassette to hold together the top plate and bottom plate with sufficient force that a series of substantially tight seals are formed around the portions of a filter membrane, sandwiched between the two plates, that are enclosed by the walls of the channels found in the top plate. Effectively, this causes the filter membrane to act as a series of strips of membrane wherein each strip is isolated from the other strips formed on the membrane by the grooves of the upper plate. In this way, the assay cassette can be employed for carrying out a plurality of reactions with reduced or eliminated cross-contamination.

In one embodiment the invention assay cassettes described herein, comprise a top plate having a frangible portion extending transversely across the top plate and a bottom plate having a peripheral side wall and being adapted to couple with said top plate to define an interior chamber capable of receiving a filter membrane. In one embodiment, the frangible portion is formed by a score extending across the width of the top plate. However, in other embodiments the frangible portion can include a cavity or a frangible seal or any other mechanism suitable for allowing the top plate to be divided into separate components. Alternatively, the top plate can include a hinge for allowing the assay cassette to be swung open for giving access to the filter membrane stored therein. The assay cassette can include a top plate that has a recessed bottom surface which extends into a chamber a distance sufficient to butt against a membrane received therein. The top plate can also include a substantially open channel that extends through the top plate and provides an opening through the top plate to allow contact with a membrane within the chamber. Accordingly, the assay cassette can include open channels that allows a sample material to be delivered through an opening in the top plate and onto a membrane contained within the chamber of the assay cassette. The open channels can be formed in a pattern, such as a plurality of longitudinally extending channels, or as a plurality of concentric rings. However, it will be understood that any suitable pattern can be employed without departing from the scope of the invention, including systems with two, four, eight, or twenty-four channels, or openings formed as dots, ellipses, wells or any other suitable configuration.

In a further embodiment, it is understood that the assay cassette includes an engagement mechanism for holding the top plate together with the bottom plate. In one embodiment, the engagement mechanism includes a catch for holding the top plate together with the bottom plate.

Optionally, the engagement mechanism allows for some vertical movement of the plates relative to each other. Accordingly, the top plate and bottom plate, in an engaged state, can be moved closer together, sandwich a filter membrane therebetween. The catch can be formed from a recessed surface in the top plate which will slidingly receive and engage with a surface protrusion located on the side wall of the bottom plate. Alternatively, the engagement mechanism can include a clasp capable of joining the top plate together with the bottom plate. In this embodiment, the clasp can be a simple elastic element, such as a rubber band that can be fitted on the outside of the cassette when the top plate and bottom plate are joined together. Other mechanisms for joining and holding together the top plate and bottom plate can be practiced without departing from the scope of the invention.

In a further embodiment, the assay cassette can include a vacuum port disposed in the bottom plate. The vacuum port can allow a vacuum to be applied to the interior chamber, and is understood to facilitate drawing fluid through the filter membrane. In one embodiment, the vacuum port is formed in part by channels formed in the bottom plate of the cassette assembly. In a preferred practice, the channels in the bottom plate are aligned with the channels in the top plate to provide for fluid to be drawn through the channels in the top plate, and the membrane and into the channels of the bottom plate, where any excess fluid can be drawn off to a waste container.

In one practice the assay cassette, when assembled, is put into a container that is part of an agitator system for performing rapid flow-through assays. One such system provides a vacuum port that may be operatively-linked to a vacuum source. Test samples and assay reagents may be applied to the filter membrane of the assay cassette through the opening in the top plate and the vacuum source can apply a negative pressure to the channels bottom plate to draw material and reagents through the membrane.

Additionally, the systems described herein can include systems for opening an assay cassette having a frangible section. These systems can include a base plate, and a top plate that is hingedly mounted to the base plate and spaced away from the base plate for defining a gap capable of receiving one end of the assay cassette. Once the assay cassette is received within the device, pivoting the top plate relative to the base plate provides a shearing force capable of

opening the assay cassette. Accordingly, it will be understood that the assay cassettes described herein can be opened in response to a shear force applied to the frangible section, wherein in one embodiment the assay cassette opens by having the frangible section of the assay cassette break-off and separate from the cassette, or alternatively, the frangible section can break along one side of the assay cassette, with the other side still attached, yet in manner that allows the two sections to move relative to each other to provide access to the interior of the cassette.

It will be appreciated that the membranes of the invention may be prepared by methods known to those skilled in the art. For example, antigens may be applied to the membranes by electrophoresis and transblotting. Antigens or other binding members may also be applied directly to membranes by, for example, pens, brushes, spray devices, ink jet devices as well as flow-through devices such as the Minislot™ (Immunetics, Cambridge, MA). It will also be appreciated that the plates and other portions of the apparatus may be made by methods known in the art, and are preferably of a solution-resistant material, *e.g.*, Plexiglas, molded plastics, aluminum, stainless steel or other synthetic materials.

Generally, according to a method of the invention, a test sample is allowed to come into contact with the surface of the membrane (on which a binding member specific for an analyte of interest is immobilized), through a multiple-channeled plate of a removable cassette of the invention. Appropriate volumes of test samples and assay reagents are placed, *e.g.*, by pipetting, on the membrane through the channels. The test sample is drawn through the membrane under vacuum. The channels may then be flushed to rinse away unbound materials. It will be appreciated that
additional reagents, indicators, or binding members can also be introduced into the channels, preferably under vacuum, to provide a detectable signal. If the test sample contains an analyte of interest, it will bind to the binding member specific for the analyte on the membrane.

The removable cassettes described herein can be provided to the consumer for a single and disposable use. The membrane provided can include any desired binding members for detection of analytes of interest, including several different binding members, to permit simultaneous screening for the presence of more than one analyte. The membrane may also include only one type of binding member, allowing detection of a desired analyte from several test samples. The

methods of the invention may also require smaller volumes of reagents than known methods and thus the apparatus and methods of the invention may provide additional costs savings. A further advantage of the apparatus and methods of the invention is the increased speed of assay as compared to conventional assay apparatus and methods. Yet a further advantage of the apparatus and methods of the invention is the increased clarity of the visual result as compared to conventional apparatus and methods.

The invention also provides kits comprising a removable cassette having a membrane with preselected binding members specific for analytes of interest immobilized thereon, and reagents for carrying out the specific binding assay. The kit may further comprise instructions for use as well as appropriate packaging.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects and advantages of the invention will be appreciated more fully from the following further description thereof, with reference to the accompanying drawings wherein;

Fig. 1 is a perspective view of an assay device having a removable cassette of a preferred embodiment of the present invention;

Fig. 2 is an exploded view of a first removable cassette of the preferred embodiment of the present invention;

Fig. 3 is an exploded view of a second removable cassette of the preferred embodiment of the present invention;

Fig. 4 is an exploded cross-sectional view of the assay device taken along lines 4-4 of FIG. 1;

Fig. 5 is an exploded cross-sectional view of the assay device taken along lines 5-5 of FIG. 1;

Fig. 6 is a top view of the assay device base plate;

Fig. 7 is an exploded view of a third removable cassette of the preferred embodiment of the present invention;

Fig. 8 is an illustration of a removable cassette encased in a sealed plastic packaging;

Fig. 9 provides an exploded view of one assay cassette according to the invention;

Fig. 10 provides a cross-sectional view of the top plate of the assay cassette of Fig. 9;

Fig. 11 depicts in greater detail the channels of the top plate of Fig. 10;

Fig. 12 depicts the underside of the top plate of Fig. 10;

Fig. 13 depicts in greater detail the latching mechanism of the assay cassette of Fig. 9;

Fig. 14 depicts in greater detail the bottom plate of the assay cassette;

Fig. 15 provides an exploded view of the assay cassette of Fig. 9;

Fig. 16 illustrates a chamber for holding an assay cassette during an assay such as a rapid flow-through assay;

Fig. 17 illustrates one system for opening the assay cassette of Fig. 9;

Fig. 18 illustrates the cassette being opened by the device of Fig. 9; and

Fig. 19 illustrates the removal of the filter of an assay cassette that has been opened.

DETAILED DESCRIPTION OF THE ILLUSTRATED EMBODIMENTS

To provide an overall understanding of the invention, certain illustrative embodiments will now be described, including an assay cassette for use during rapid flow-through assaying, that includes a frangible section. However, it will be understood by one of ordinary skill in the art that the assay cassettes described herein can be adapted and modified to provide systems that can be employed in other types of assays, including any assay that requires filtering a sample material through a filter membrane, such as direct or sandwich assays.

In such assays, an analyte is bound to a binding member immobilized on the membrane, and a labeled second binding member is bound thereto to provide a detectable signal. Any variety of labels or indicator schemes which provide a detectable signal that analyte binding has occurred can be employed with the systems described herein, including, for example, direct labels such as fluorescent, radioactive and chromophoric labels. Labels which may require development or enzymatic reagents, such as horseradish peroxidase or alkaline phosphatase, can also be utilized. Additionally, indirect label vehicles such as Protein A or avidin/biotin methods, know to those skilled in the art, can also be adapted for use with the apparatus and methods described herein. Accordingly, it will be understood that the systems and methods described herein are exemplary

and provided for purposes of illustrating the invention and that many modifications, substitutions, and additions can be made to the systems described herein without departing from the scope of the invention.

In a typical multi-step binding assay, each reagent or wash solution is added sequentially as soon as the previous solution has been fully aspirated through the membrane. For example, in detecting antibodies reactive with antigens on a Western Blot, the sequence of solutions generally comprises, in order: blocking solution (*e.g.*, detergent Tween-20), primary antibody (diluted human serum), three washes with buffer containing phosphate-buffered saline and Tween-20 detergent, secondary antibody (*e.g.*, diluted anti-human IgG-alkaline phosphatase conjugate), three more buffer washes, distilled water, and enzyme substrate (*e.g.*, 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium solution). Given the rapidity of aspiration through the membrane, processing with all solutions can be completed in about 10 minutes. This method is thus 10-20 times faster than the conventional processing method.

The assay device and binding assay of the invention are well-suited for conducting immunoassays for the presence of analytes in any biological material, *e.g.*, an antibody or antigen analyte in human or animal serum, urine, stool, saliva or other body fluids, secretions or excretions. It will be appreciated, however, that the device and assays of the invention can be adapted to utilize the binding affinity of any binding member such as, for example, haptens, complimentary nucleic acid sequences, ligands, small molecules and receptors. The invention can also be used to screen for the presence of microbial organisms, including bacteria, viruses and fungi. The device of the invention can additionally be used to screen or otherwise characterize binding specificities of monoclonal antibodies, antibodies of different species and antibodies produced by genetic engineering or other *in vitro* techniques.

Examples of binding assays which can be conducted with the device and in accordance with the principles of the invention include assays to detect the presence of antibodies specific for bacterial proteins of *Borrelia burgdorferi* which causes Lyme disease, for viral proteins of HSV which causes herpes, for viral HIV proteins implicated in acquired immunodeficiency syndrome (AIDS), for antigens specific to human chorionic gonadotropin (HCG) to detect pregnancy, for rheumatoid arthritis, and for a variety of bacterial and viral infections. It is also

contemplated that the device and assays of the invention be used to screen for toxins such as, *e.g.*, that of *Clostridium difficile*, and to screen for specific nucleic acid sequences to detect, *e.g.*, pathogens, genetic defects, etc.

The invention also provides kits comprising a top plate with channels and a membrane with preselected binding members specific for analytes of interest immobilized thereon, and packaging enclosing the top plate and membrane. Appropriate processing reagents for carrying out the specific binding assay may also be included, as well as instructions for use. To initiate the test the operator would need only to connect the disposable unit to a vacuum source and apply test solutions to the channels. Application of test solutions could be performed manually, or by a robotic liquid handling system under software control. In all cases, a visual or otherwise detectable pattern of reactivity, in the form of bands or otherwise, is generated in each test channel, and can be analyzed to obtain a test result.

Fig. 1 shows a perspective view of an assembled assay device 20 of a preferred embodiment of the present invention. The assay device 20 generally has a base plate 22, a cover plate 24, and a removable cassette 26 for receiving assay samples. As can be seen, the cover plate 24 and base plate 22 are generally rectangular in shape, although their size and shape can vary provided that the removable cassette 26 is suitably accommodated. The base plate 22 and cover plate 24 are preferably formed from a solution-resistant material such as, but not limited to, plastic, aluminum, stainless steel or other synthetic materials. In practice, and as illustrated, the removable cassette 26 may be disposed between the base plate 22 and cover plate 24, with the cover plate 24 having an aperture so as to expose the sample introduction channels 25 of the removable cassette 26.

As will be subsequently described in further detail, the base plate 22 has a central pocket (not shown) and a vacuum/aspiration port 36. The central pocket of the base plate 22 serves as a collector for liquid aspirated through a membrane component of the cassette 26. The vacuum/aspiration port 36 is connected via a tubing adapter 38 to a length of tubing (not shown) leading to a trap (not shown) and ultimately to a vacuum source (not shown). In practice, test samples and assay reagents are applied to the membrane through the channels of the cassette 26 under vacuum.

It should be understood that the cover plate is not essential for the proper operation of the assay device 20. The cassette 26 may be held in the base plate with the application of pressure.

In view of this, small actuated clips or cam fasteners on the base plate may be used to apply the pressure needed. Furthermore, the presence of the vacuum that is applied to the central pocket of the base plate 22 may be sufficient pressure in and of itself.

Fig. 2 presents an exploded view of a removable cassette 28 of a first preferred embodiment of the present invention. The removable cassette 28 has a top plate 30, membrane 34, and an optional wicking member 36.

The top plate 30, preferably made by machine processing or injection molding of, for example, acrylic, styrene plastic or polymethylpentene, has a channel portion 29 that is skirted by a flange portion 31. The top plate 30 also has an array of eight parallel channels 32 projecting perpendicularly from the top surface of the channel portion 29. It will be appreciated however, that the plate may have one or more channels. The channels 32 project completely through the top plate 30 and are preferably 10 centimeters long by 0.35 centimeters wide. With the eight channel configuration, up to eight samples may be received for assay. However, it should be readily understood that the number of channels may be increased or decreased to provide the ability to receive a greater or lesser number of specimens, and the channel dimensions may also vary depending upon the assays for which the particular cassette is designed.

The channels 32 are relatively long and narrow with very little structure over the channels obstructing the ability to apply solutions to the entire channel length (*i.e.*, the channels 32 are open at the top surface 33 of the channel portion 29). Furthermore, even if the channels 32 are partially or completely covered, the depth of the channels is great enough so that liquid applied to the channels will be overlayed by air.

The membrane 34 of the removable cassette 28, preferably a nitrocellulose membrane generally cut to a rectangular or square shape, is affixed to the bottom surface of the top plate 30 with the wicking member 36, such as filter paper, optionally connected to the membrane 34 on the side of the membrane 34 opposite the membrane surface that is attached the top plate 30. The wicking member 36 provides a more uniform distribution of liquid through the membrane. The membrane 34 (and optionally the wicking member 36) may be affixed to the top plate 30 by welding, *e.g.*, ultrasound or vibration welding. This welding provides a seal between the channels 32, such that a sample applied to one channel does not diffuse laterally through the membrane to a neighboring channel.

The membrane 34 can include channel numbers, letters, or other designations embossed, imprinted, stamped, or otherwise affixed thereon to assist the user in proper channel identification.

Alternatively, the top plate 30 of the removable cassette 28 may be embossed, imprinted, stamped, or otherwise designated to assist in identifying cassette channels. The numbering or lettering scheme can include every channel, every other channel, or any other convenient system.

The membrane 34 provides a platform on which a binding member specific for the analyte is immobilized. Typically, the membrane comprises a flexible, non-woven paper-like sheet of material such as nitrocellulose, nylon, and polyvinyldifluoridene (PVDF), or other materials having similar immobilizing properties, such as a covalent coupling membrane. In one preferred embodiment, proteins or nucleic acids are electrophoretically separated on a gel and transferred onto the membrane using Western, Northern or Southern blotting techniques well know to those skilled in the art. It should be appreciated, however, that a binding member can be immobilized on the membrane in any number of ways, including electrophoretically, chemically or physically.

Fig. 3 presents an exploded view of a removable cassette 40 of a second preferred embodiment of the present invention. This cassette 40 has a membrane 52 disposed between a top plate 44 and a bottom plate 46. The top plate 44 and bottom plate 46, preferably made with acrylic, styrene plastic or polymethylpentene, each have 8 channels (48,50) that project perpendicularly from the top surface of a plate (43,45) through to the bottom surface of the respective plate (47,49). A wicking member 42 may be placed between the membrane 52 and the top plate 44 or bottom plate 46.

When assembled, the two plates (44,46) are held together with a suitable fastening means, sandwiching the membrane 52 (and the wicking member 42). Suitable fastening means are known in the art and include press-fit locating pins, tape, or a hinge and latch assembly, preferably located on flange portions of the plates.

Referring to Fig. 4, an exploded cross-sectional view is presented taken along lines 4-4 of the assay device 20 holding the removable cassette 40 of Fig. 3. The cassette 40 may be disposed between the cover plate 24 and base plate 22 with the cover plate 24 having an aperture allowing the exposure of the top plate channels 48 of the removable cassette 40.

The cover plate 24 has a groove 56 that receives a flange portion 58 that skirts the channel portion of the top plate 44. The base plate also has a groove 60 that receives a flange portion 62 that skirts a channel portion 63 of the bottom plate 46. As previously indicated, the base plate 22 has a central pocket 65 and vacuum/aspiration port 36 with tubing adaptor 38 that can be operatively linked to a vacuum source (not shown). With this assembled assay device 20, test

samples and assay reagents may be applied to the membrane 42 through the multiple channels (48,50) of the removable cassette 40, and drawn through the membrane 42 under vacuum.

Fig. 5 shows an exploded cross-sectional view of Fig. 1 taken along lines 5-5. As can be seen, the base plate 22 and cover plate 24 contain fasteners (64,66) that are used to lock the assembly 20. The threaded end (68,70) of each fastener is inserted through apertures (72,74) in the cover plate 24, and threadably mated with recessed cavities (76,78) formed in the base plate 22. In order to assist in proper alignment of the cover plate 24 and base plate 22 during fastening, locator pins (82,84) are provided that extend from the base plate 22 and that are received by alignment apertures (86,88) in the cover plate 24. It will, however, be appreciated that other means for fastening or clamping the plates (22,24) of the device 20 are readily available.

The heads of the fasteners (64,66) are preferably large enough to permit easy manipulation by hand and are also knurled, ridged, or otherwise textured to provide a good gripping surface. The fasteners (64,66) can be solid or hollow, but are constructed of inert rigid materials which are resistant to the solutions and reagents used in the assay. Suitable materials include both metal and plastics, preferably acetal plastics.

Referring to Fig. 6 and as previously indicated, the base plate 22 has a central pocket or cavity 118 that becomes a vacuum chamber when a cassette is placed over the cavity 118 so as to cover it. A gasket 120 of soft rubber or other seal material is placed on the groove 122 in the base plate 22 that runs around the periphery of the cavity 118. The flange portion of the removable cassette rests on this gasket 120 and pressure applied by the vacuum or cover plate effectively seals the cassette and base plate together. The preferred embodiment utilizes a gasket material that is a sponged rubber sheet, however it can be seen that an O-ring gasket or many other materials and configurations may be used to create the seal. As previously indicated, only a small amount of pressure is needed to be applied to the cassette for proper operation of the assay device. With the proper gasket material, a seal may be created by lightly pressing on the cassette, with the subsequent application of the vacuum maintaining the seal during assaying. Once the chamber seal is created, application of the vacuum draws the applied liquids through the channels of the cassette. In order to assist in the collection of filtered liquid in the cavity 118, grooves 124 are placed in the cavity floor, effectively routing the collected liquid to the vacuum port for evacuation.

Fig. 7 illustrates an alternate embodiment of the present invention. This cassette 90 is preferably used in an automated instrument as the components are ultrasonically welded to form

a unitary cartridge. The cassette 90 has a cassette top 92, membrane 94, optional wicking member 96, support 98, and cassette bottom 100. As with the previously described cassettes, the cassette top 92, support 98 and cassette bottom 100 are formed from a solution resistant material such as plastic, aluminum, stainless steel or other synthetic material.

The support 98 has a top side 104 with channels 106 corresponding to the channels 102 of the cassette top 92. The support also has multiple footings 108 for operative contact with the cassette bottom 100 which has multiple grooves 110 aligned with the channels 102 of the cassette top 92. The cassette bottom 100 also has a vacuum/aspiration port 112 for connection to a vacuum pump (not shown).

The membrane 94 is disposed between the cassette top 92 and support 98. As with the other cassette embodiments, a wicking member 96 such as filter paper, may be included in the cassette 90, and is preferably inserted between the membrane 94 and support 98. The support 98 is inserted into the cassette bottom 100, and all the components of the cassette are interlocked and sealed.

In operation, solutions are introduced into the channels of the cassette top, contacting the membrane, and analytes of interest (if present) will bind to the binding members immobilized on the membrane 94. The binding members may also be pre-introduced to the membrane prior to operation.

The various embodiments of the cassette as previously described can be provided to the consumer as a preassembled unit. As shown in Fig. 8, an assay device having a removable cassette 114 can be supplied in a sealed plastic packaging 116. This packaging 116 can include separate moisture-proof packaging to avoid contamination and permit pre-moistening of the membrane, thereby promoting longevity of the package contents. It will be appreciated, however, that the components of a cassette of the present invention can also be provided individually or as a complete assay device. It will also be appreciated that, although the various components of the apparatus of the invention can be cleaned and reused, the entire cassette unit or any of its components can be discarded after a single use, *i.e.* disposable.

The following Specific Example illustrates practice of the invention. The example is for illustrative purposes only and is not intended in any way to limit the scope of the claimed invention.

SPECIFIC EXAMPLE

Lyme Disease IgG Antibody Detection

The following Lyme disease IgG antibody detection assay was performed with the apparatus of the present invention.

A nitrocellulose membrane bearing electrophoretically resolved antigens of *Borrelia burgdorferi* was prepared by standard blotting procedures (*e.g.*, Towbin, H., Stehelin, T. and Gordon, J., *Proc. Nat. Acad. Sci. U.S.A.* 76:4350-4354 (1979)), then washed 30 minutes in distilled water. Additional stripes of defined antigens may be applied to the membrane at this point by non-electrophoretic methods. The nitrocellulose membrane was 0.2 μ pore size and was obtained from commercial suppliers such as Schleicher & Schuell (Keene, NH), Whatman, Inc. (Fairfield, NJ), or others.

The nitrocellulose membrane was cut to fit a cassette, 2" wide x 4" long. The wet membrane was placed over an identically sized piece of dry filter paper. As described herein, a variety of filter papers may be used, but a paper specifically intended for wicking is optimal. #320 paper from Ahlstrom Filtration Inc. (Mount Holly Springs, PA), a 2.5 mm thick blotting paper, was used.

The membrane and filter paper were placed in the removable cassette, the cassette top and bottom were fixed together, and the membrane was allowed to dry. Drying of the membrane was preferable for packaging, in that a wet membrane could support the growth of microbes during shipping and long-term storage, in the absence of sterilization. However, it will be appreciated that membranes may be used wet.

The cassette was mounted on a base fixture and the screws were tightened. Tightening of the screws applies pressure to the cassette, thereby sealing the membrane against the cassette channels and preventing cross-channel leakage of solutes, and also seals the cassette against the vacuum chamber, preventing vacuum leakage.

The entire unit was placed on a vertical rocking platform, and all subsequent steps (liquid additions, incubations and aspiration) were performed while the unit rocked at approximately 100 cycles/minute through an 8° angle.

A wetting solution consisting of phosphate-buffered saline (PBS) containing 0.5% Tween-20 detergent was introduced into the cassette channels. When a dry membrane is enclosed in the cassette, this wetting step is essential to provide for even aspiration of succeeding solutions through the membrane in each channel. Enough wetting solution must be applied to thoroughly wet both the membrane and the underlying filter paper, *e.g.*, 1-2 ml per channel. The solution was left to permeate the membrane and wet it by capillary action for 3 minutes, then the vacuum was

turned on and the remaining solution drawn through the membrane. Once all solution was cleared, the vacuum was turned off.

The test sample was then added. Typically, 20 µl of a human serum (*e.g.*, from a patient suspected of having Lyme disease) is mixed with 600 µl of PBS/0.5% Tween-20 before introduction into the cassette. Diluted serum specimens were incubated in the channels for 3 minutes, then vacuum was turned on and solutions were drawn through the membrane in about 10 secs. The membrane was subjected to 3-4 washing steps. In each one, approximately 600 µl of PBS/0.5% Tween was added per channel with the vacuum on constantly.

The detection reagent was then added. In one procedure, an affinity purified goat anti-human IgG-alkaline phosphatase conjugate (commercially available from Jackson Immunoresearch, West Grove, PA) was diluted 1000-fold in PBS/0.5% Tween-20/0.05% Polyvinylpyrrolidone (MW 360,000), and 600 µl were added per channel. Following a two-minute incubation, the vacuum was turned on and all conjugate solution aspirated through the membrane within about 10 secs. The addition of polyvinylpyrrolidone to the conjugate dilution buffer increased the sensitivity of detection of bound antibody without a parallel increase in background noise; it thereby functioned as a reaction "enhancer".

The membrane was subjected to 3-4 washing steps. In each one, approximately 600 µl of PBS/0.5% Tween-20 was added per channel with the vacuum on constantly. The membrane was then washed with about 600 µl distilled water per channel.

The enzyme substrate, a solution containing 4-chloro-3-bromo-2-indolyl phosphate and nitroblue tetrazolium (commercially available from Kierkegaard & Perry, Gaithersburg, MD) was added. To each channel, 600 µl undiluted substrate was added and incubated 3.5 minutes, after which vacuum was turned on and solution aspirated through the membrane in about 10 secs. The membrane was then washed twice with distilled water, each time by adding approximately 600 µl per channel with constant vacuum aspiration.

Results were visualized on the membrane surface in each channel as purple bands. For Lyme disease, specific patterns of bands can be interpreted as a positive result. For ease in analyzing the pattern of bands, the cassette can be removed from the base unit and disassembled at this point, and the membrane removed from the cassette. The membrane can be dried to provide a permanent record. Alternatively, the membrane can be scanned, even while still within the cassette, by a scanning device such as a CCD camera, which can then generate a permanent electronic record of the results.

A colloidal gold conjugate (*e.g.*, colloidal gold - anti IgG conjugate) may be substituted for the enzyme conjugate. The resulting bands on the membrane are colored pink rather than purple, but the sensitivity of detection of the bands is otherwise equivalent. The enzyme conjugate method described above may be followed, wherein the conjugate is added, the membrane is washed and then the substrate is added and washed again. By utilizing the colloidal gold conjugate, the steps of the addition of substrate and following washes may be eliminated, reducing the total assay time by about 5 minutes, or about 33%, to about 10 minutes total. A preferred gold conjugate is 40 nm colloidal gold coated with anti-human IgG antibodies (commercially available from British Biocell, Cardiff, U.K. and Amersham, Arlington Heights, IL). The colloidal gold conjugate may be diluted to approximately 1-2 $O.D._{520}$ units with PBS/0.5% Tween-20 and applied as the conjugate. The membrane may then be washed several times as set forth in the above method. Pink bands may then be visualized on the membrane.

Fig. 9 depicts another embodiment of a cassette assembly according to the invention. Specifically, Fig. 9 depicts an assay cassette 210, having a label 212, a top plate 214, a membrane 216, a filter pad 218, and a bottom plate 220. The cassette 210 depicted in Fig. 9 is a disposable device, typically manufactured for one-time use and made out of plastic components which are readily manufactured by techniques well known in the art.

The exploded view of Fig. 9 shows the relative order in which the above elements can be assembled together to provide an assay cassette suitable for rapid flow-through binding assays. Specifically, Fig. 9 shows that the label 212 can attach to the top plate 214 and that the top plate 214 can fit over the bottom plate 220 so that the membrane 216 and the filter pad 218 fit within an interior chamber that is formed within the assay cassette 210 when the top plate 214 is fit over the bottom plate 220. Accordingly, the elements depicted in Fig. 9 cooperate to form an assembly that can securely hold a membrane 216 between the two plates of the cassette. Moreover, as explained in greater detail hereinafter, the top plate and bottom plate can be assembled in a manner that allows the plates to be moved closer together, for compressing the membrane 216. This cassette assembly can then be employed within a device for performing a rapid flow-through assay, such as the devices described with reference to Fig. 8.

The label 212 depicted in Fig. 9 is a plastic label having a two-part adhesive backing which allows the label 212 to be affixed to the upper surface of the top plate 214. To this end, the underside of the label 212 can have an adhesive material applied around the rim of the label 212 such that the label 212 can seal to the perimeter of the top plate 214. In this embodiment, the

adhesive material is away from the channels 232, and therefore away from the sample and reagent materials. The portion of the label 212 that fits over the channels 232 can be water proof, or other wise fluid resistant. In the embodiment of Fig. 9, the label 212 includes a sample/viewing window 222 disposed at one end of the label 212. The sample/viewing window 222 is an aperture extending transversely along one end of the label 212 and providing an aperture through which a sample material can be delivered into the cassette assembly 210. The depicted label 212 includes three peel-away labels 24A-24C. Each of these three labels provides a set of data fields into which a lab technician can enter information. The information can include the patient identifier, such as the patient's name or social security number, the date of performing the assay, a barcode describing information about the assay, patient or lab, or any other relevant information. Each of the three peel-away labels 224A-224C can be employed by the lab technician for labeling different components that are employed during the assay. For example, one label can be left on the cassette device 210, or located to a different location on the cassette device 210. A second label can be maintained in-house, such as in an internal lab notebook. And the third peel-away label can be employed for labeling a kit that includes the processed portions of the assay, such as a processed membrane. The depicted label 212 is waterproof, or otherwise fluid resistant for both top and bottom. In alternate embodiments, the label 212 can be a multi-part label, wherein individual parts of the label can be peeled from the multi-part label and applied to different components of the test kit.

The top plate 214 depicted in Fig. 9 includes a side wall 228 that surrounds the periphery of the plate 214. In the top surface 230 of the top plate 214, there are a plurality of channels 232 that extend longitudinally across the top surface 230. In the depicted embodiment there are eight channels 232, however any number of channels can be employed depending on the application. The label 212 fits between the walls of a raised lip 235 which extends around three sides of the top surface of the top plate 214. The depicted top plate 214 further includes a weakened portion 234 that in the depicted embodiment comprises a score disposed at one end of the top plate 214 and extending for the full width of the top plate 214 and being transverse to the grooves 232.

Fig. 10 depicts in greater detail a portion of the top plate 214. Specifically, Fig. 10 provides a cut-away view and side perspective of one end of the top plate 214. Specifically, Fig. 10 shows in greater detail the score 234 that extends transversely across the top of the top plate 214. The cut-away perspective of Fig. 10 shows that the top plate 214 has a recessed bottom wall 238 and has a catch wall 240 that has a plateau 242 that, as will be described in greater detail

hereinafter, provides a surface for engaging against a catch on the bottom plate 220 depicted in Fig. 9 to engage the bottom plate 220 with the top plate 214.

Fig. 10 also illustrates in greater detail that the score 234 is formed as a V-shaped groove that extends partially into the top plate 214. It will be understood that this groove provides a weakened section along the top plate 214 along which a fracture can more easily occur. In particular, it is understood that a mechanical force, such as a shear force applied to the top plate 214 can result in a fracture occurring along the grove 234 such that the fracture travels downward from the grove 234 to the recessed bottom surface 238. This results in the fracture of the top plate 214, fracturing into two components, a larger component and a smaller component. Although in the depicted embodiment, the weakened portion is depicted as a score that extends across the width of the top plate 214. It will be understood that the weakened portion 234 can also include a concavity extending along the width of the top plate 214 wherein a fracture is more easy to occur, a breakable seal that can be pulled away by a lab technician to separate the top plate 214 into a larger component and a smaller component, or any other mechanism suitable for providing a weakened portion within the top plate 214. Other embodiments can include notches that are square, or V-shaped, thin wall molded sections that in molding would provide internally molded knits or stress lines, or any other structure susceptible to breakage. It will further be understood that although the weakened portion 234 is depicted as being in the top surface of the top plate 214 at one end of the top plate 214, it will be understood that the weakened portion 234 can be located at alternative positions, including at the other end of the top plate 214, close to the center of the top plate 214, at an edge of the top of 214 between the wall 214 and the side wall 228, on the underside of the top plate 214, in the side walls, or any other suitable location. In other embodiments, the cassette can be made with clasps that allow a distal end of the assembled cassette to be separated from a proximal end of the cassette, therefore, providing a system wherein a section of the assembled cassette is detachable from the rest of the casette.

With reference to Fig. 11, the channels 232 and the top plate 214 can be described in greater detail. The channels 232 depicted in Fig. 11 are open grooves that extend longitudinally and that provide openings from the top surface 230 of the top plate 214 through to the bottom surface 238 of the top plate 214. Accordingly, the channels 232 provide openings through which a substance can be delivered into the cassette assembly 210. In the depicted embodiment the channels 232 are alternately staggered to provide for greater visibility as the lab technician fills each of the grooves with sample material to be processed during the assay. It will be understood

that each of the channels 232 can receive a separate sample of material to be assayed. It will further be understood that the channels 232 are fluidicly isolated from each other such that fluid cannot pass through the walls of the channels 232 to leave one channel and to enter into another channel. It will also be understood that the channels can be provided by other elements, including dots, and wells, similar to the dots found on a micro-titer plate, grooves, transverse channels or combinations thereof.

Fig. 12 depicts in greater detail the bottom surface 238 of the top plate 214. Specifically, Fig. 12 depicts that the bottom surface of 238 is recessed from the top of the top plate 214 and extends into the chamber defined by the top plate 214 and the side wall 28. Additionally, Fig. 12 depicts that each of the channels 232 extends all the way through the top plate 214 to define a plurality of openings in the bottom surface 238. Fig. 12 depicts that the catch wall 40 is formed into the side wall 228 of one end of the top plate 214, however, it will be understood that a similar catch wall 40 is formed in the other sidewall of the cassette. As shown in Fig. 12 the catch wall 240 is centrally located and extends for a portion of the end of the side wall 228. The end of the side wall 228 in which the catch wall 240 is located is, in the depicted embodiment, offset from the rest of the side wall 228. Specifically, the side wall 228 steps inward toward the recessed bottom surface 238 as the wall 228 extends towards the catch wall 240. This is understood to provide one end of the top plate 214 with a reduced width. This reduced width feature can be employed for orientation purposes, and specifically to allow the narrowed end of the top plate 214 to be received within a device for fracturing the top plate 214. Such a device will be described in greater detail hereinafter.

Fig. 13 depicts in greater detail the engagement mechanism of the cassette assembly 210. Specifically Fig. 13 provides a cross-sectional view of the cassette assembly, showing the engagement mechanism that is formed by the catch wall 240 located in the end wall 228 of the top plate 214 and by the lock wall 244 located on the end wall of the bottom plate 220. The locking wall 244 includes a lip 248 that extends outwardly from the side wall of the bottom plate 220. The lip 248 can fit into the chamber defined by the catch wall 240 that is notched into the side wall 228 of the top plate 214. Upon insertion of the locking wall 244 into the cavity formed above the catch wall 240, the lip 248 extends into the cavity and butts against the catch wall 240, which acts as a mechanical stop preventing the lip 248 from moving downwardly. Above the lip 248, is a gap 246 that allows the top plate 214 to travel downward toward the bottom plate 220 and compress the membrane 216 held between the top plate and the bottom plate. Accordingly,

upon insertion of the locking wall 244 into the cavity formed above the catch wall 240, the bottom plate 220 is stopped from moving downwardly and away from the top plate, thereby being engaged therewith, however, the top plate 214 can be move downward toward the bottom plate 220 to allow further compression of the membrane 216. Such movement can occur when the assay cassette 210 is placed within a container, such as that described hereinafter with reference to Fig. 16, and the top plate of the container is brought down and pushed against the top plate 214 of the assay cassette.

Although the assay cassette 210 has been depicted having the locking walls 248 and catch wall 240 as the engaging mechanism, it will be understood that other devices can be employed for engaging the top plate 214 with the bottom plate 220. For example, a clasp mechanism can be employed which can wrap around the exterior of the assay cassette 210, wrapping around both the top plate 214 and the bottom plate 220 and thereby preventing the plates from separating. Similarly, a screw mechanism can be employed for allowing a screw to be threaded through both the top plate 214 and the bottom plate 220, thereby joining the two plates together. Gaskets can be applied around the periphery of the paltes, to allow the plates to seal and to allow further compression of the plates, and the membrane 216. Additionally, other techniques can be employed, such as ultra-sonic welding, fast drying glues and adhesives, which can be applied in various ways for permanently joining the top plate 214 to the bottom plate 220, and other mechanisms can be employed for allowing the plates to be brought closer together and for allowing the membrane 216 to be compressed.

Fig. 13 further depicts the membrane 216, and filter pad 218, which are disposed between the top plate 214 and the bottom plate 220. In particular, Fig. 13 shows the filter pad 218 supports the membrane 216, and provides a raised surface upon which the membrane 216 can be seated. Fig. 13 further depicts that the coupling of the bottom plate 220 with the top plate 214 forms an interior chamber 15 in which are disposed the membrane 216 and the filter pad 218. The filter pad 218 is dimensionally adapted to raise the membrane 216 into engagement with the recessed bottom surface 238 of the top plate 214 when the top plate 214 is joined to the bottom plate 220. In this way, the membrane 216 is sandwiched within the assay cassette 210.

The membrane 216 can be a nitrocellulose membrane bearing electrophoretically resolved antigens of *Borrelia burgdorferi* was prepared by standard blotting procedures (*e.g.*, Towbin, H., Stehelin, T. and Gordon, J., *Proc. Nat. Acad. Sci. U.S.A.* 76:4350-4354 (1979)), then washed 30 minutes in distilled water. Additional stripes of defined antigens may be applied to the membrane

at this point by non-electrophoretic methods. The nitrocellulose membrane can have a 0.2 μ pore size and can be obtained from commercial suppliers such as Schleicher & Schuell (Keene, NH), Whatman, Inc. (Fairfield, NJ), or others. The nitrocellulose membrane can be cut to fit the cassette, such as being cut to 2" wide x 4" long. The wet membrane can be placed over an identically sized piece of dry filter paper. A variety of filter papers may be used, but a paper specifically intended for wicking is optimal, such as. #320 paper from Ahlstrom Filtration Inc. (Mount Holly Springs, PA), a 2.5 mm thick blotting paper.

It will further be noted that in the embodiment depicted in Fig. 13, the bottom plate 220 includes a weakened portion 52 that, when the top plate 214 is joined to the bottom plate 220 is positioned in opposition to the weakened portion 234 of top plate 214. This alternate embodiment allows a shear force to fracture the top plate 214 and the bottom plate 220.

Fig. 14 depicts in greater detail the bottom plate 220. Specifically, Fig. 14 depicts the bottom plate 220, the side wall 60, alignment pins 62, bottom surface 64, vacuum slots 68, and the locking wall 244.

The side wall 60 forms a peripheral wall around the bottom plate 220, running along the full perimeter of the bottom surface 64. Along the internal surface of the side wall 60 are a plurality of alignment pins 62. The alignment pins 62 are mounted to the side wall and dimensionally adapted to extend into the interior of the bottom plate 220 a distance sufficient to allow for proper alignment of the filter pad 218 and filter16 that are disposed within the bottom plate 20. Fig. 14 further depicts the locking wall 244 that is formed integrally into one part of side wall 60. Specifically, the depicted locking wall 244 is formed by two groves 70 that extend through the side wall 60. Accordingly, the locking wall 244 lacks side wall support and is anchored only at the bottom section of locking wall 244. Accordingly, as the material of the bottom plate 220 is a somewhat resilient molde or machined plastic material, the side wall 244 can deflect laterally, either inwardly or outwardly relative to the interior of the bottom plate 220. Fig. 14 further depicts that the locking wall 244 has a outwardly extending protrusion that forms the lip 248. This outwardly extending protrusion causes the locking wall 244 to deflect inwardly as the bottom plate is being slid into the top plate. However, upon reaching the cavity above the catch wall 240, the resilient locking wall 244 drives the protrusion into the cavity causing the lip 248 to butt against the locking wall 240 engaging the bottom plate with the top plate.

Fig. 15 provides an exploded view with an upward looking prospective of the assay cassette 210 depicted in Fig. 9. Specifically, Fig. 15 shows the assay cassette 210 which includes

the label 12, the top plate 214, the membrane 216, the filter pad 218 and the bottom plate 220. From the prospective provided by Fig. 15 it can be seen that the recessed bottom surface 238 of the top plate 214 is brought down upon the membrane 216 to sandwich the membrane 216 and filter pad 218 between the recessed bottom surface 238 and the bottom plate 20.

Fig. 15 also illustrates the bumps 278 that are optionally provided on the bottom surface 276 of the bottom plate 220. Specifically, the bumps 278 are located on the ribs 236 between the channels 68 that extend through the bottom plate 220. The bumps 278 on the ribs 236 help push the filter pad 218 against the bottom surface of the top plate 214, by allowing the vacuum applied to the underside of the cassette to act on the cassette and pull down the membrane and filter pad. In the depicted embodiment there are two sets of bumps 278, however, any suitable number and pattern can be employed to provide for optimal pressure on the filter pad. The depicted bumps 278 include an upper set and a lower set, each of which is longitudinally spaced apart. Together the pairs of bumps provide a gap between the bottom surface 276 of the cassette 210 and the bottom surface of the chamber in which the cassette 210 can be received during a rapid flow through assay. One such chamber is described in the above referenced patent application entitled "Systems and Method for Rapid Blot Screening", and another chamber is described with reference to Fig. 16. The gap provided by the bumps 278 allows for a negative pressure within the interior of the chamber holding the assay cassette 210 to be applied through the grooves 68 to the underside of the filter pad 218 and membrane 216. In this way, a negative pressure applied to the chamber holding the assay cassette 210 can act on a sample material applied through the sample window 220 in label 12 to pull sample material through the membrane 216 and filter pad 218 and to be drawn through the grooves 68.

One such chamber for holding the assay cassette 210 is depicted in Fig. 16. Specifically Fig. 16 illustrates a chamber 280 that includes a lid 282 that is hingedly mounted to a base plate 284, and then further includes a label window 288 and a pipette window 290. As illustrated by Fig. 16 the assay cassette 210 can be received within the chamber 280 to be held there during the rapid flow through assay. The lid 282 can seal against the base plate 284, wherein the base plate 284 can have a perimeter gasket that allows a vacuum seal to be formed between the lid 280 and the base plate 284. Similarly a gasket seal can fit above the perimeters of the underside of the label viewing window 288 and the pipette window 290. This allows the lid 280 to form vacuum seals against the assay cassette 210, while still allowing a lab technician to view the label on the

assay cassette 210 and to access the channels of the assay cassette 210. In an alternative embodiment, each of the windows comprises a transparent surface located within the body of the lid 280. The transparent servers provides a viewing structure that allows for viewing of the label 212, and for viewing of the channels of the assay cassette 210. In other embodiments of the chamber 280, no viewing windows are provided. The chamber 280 can further include a vacuum port (not shown) that can be disposed in the bottom plate 284 and that can be employed for applying a negative pressure to the interior of the chamber 280. As discussed above with a reference Fig. 15 this negative pressure can draw sample and other fluid material through the membrane 216 for performing the flow through assay.

In one embodiment, the base plate 284 includes a perimeter gasket against which an assay cassette received within the chamber 280 can sit. The gasket provides a vacuum seal that forms above the perimeter of the bottom surface 276 of the bottom plate 220 of the assay cassette 210. A vacuum port (not shown) in the bottom surface of the bottom plate 284 can be aligned to apply a negative pressure against the bottom surface 276 of the assay cassette 210. The chamber gasket seal between the assay cassette 210 and the bottom plate 284 of the container 280 provides a vacuum tight chamber that can maintain the negative pressure so that it is transmitted to the sample material being processed in the assay cassette 210.

After the assay cassette 210 has been processed such that sample material has been filtered through the membrane 216 held by the assay cassette 210, the assay cassette 210 can be opened to allow a lab technician to remove the membrane 216 for subsequent processing and analysis. To this end, the cassette 210 can be placed in an opening device, such as the device 210 depicted in Fig. 17. As shown in Fig. 17 the device 100 can include an upper plate 302 that is hingedly mounted to a lower plate 304, by the hinge 308. For the illustrated embodiment, the upper plate 302 is spaced away from the base plate 304 a distance sufficient to allow the assay cassette 210 to fit within the gap formed between the two plates. For the depicted embodiment the assay cassette 210 fits within the gap and comes to rest against the arm 310 that acts as a stop to prevent further movement of the assay cassette 210. The arm 310 is disposed on the upper plate 302 a distance sufficient to dispose the groove 234 at a point at which sheer forces will act when the upper plate 302 is pivoted downwardly toward the base plate 304.

As shown by Fig. 18 pivoting the upper plate 302 toward the base plate 304 causes sheer forces to act on the groove 234 allowing the one end of the assay cassette 210 to break off from the other end. In the embodiment depicted in Fig. 18 the portion of the assay cassette 210 that

breaks off 316 is allowed to fall away from the device 300 and can land into a container, such as a jelly container, that can be adapted for collecting waste product that can be disposed of properly later. In the depicted practice, the entire end of the cassette is separated from the cassette, although in other embodiments, only a portion of the top section is removed.

Fig. 19 depicts that once the end portion of the assay cassette 210 is fractured and removed from the larger component of the assay cassette 210, the filter paper can be removed from the interior chamber. To this end Fig. 19 shows that the top plate 214 can be slid back to unlatch the top plate from the bottom plate. In a subsequent step the lab technician can lift the cover from the bottom plate exposing the membrane. Any additional steps that are necessary can be performed at that time and after performing such steps the lab technician can remove the membrane and filter medium from the bottom plate for further analysis or for disposal.

Those skilled in the art will know or be able to ascertain using no more than routine experimentation, many equivalents to the embodiments and practices described herein. It will also be understood that the systems described herein provide advantages over the prior art including improved ease of processing. Accordingly, it will be understood that the invention is not to be limited to the embodiments disclosed herein, but is to be understood from the following claims, which are to be interpreted as broadly as allowed under the law.

We claim:

1. A top plate for a disposable assay cassette used in flow-through assaying, comprising:

a first top plate surface;

a second top plate surface; and

a substantially open channel projecting from the first top plate surface to the second top plate surface.

2. The top plate of Claim 1 further comprising a flange that skirts a portion of the top plate that contains the substantially open channel.

3. The top plate of Claim 1 wherein the top plate has a plurality of substantially open channels.

4. The top plate of Claim 1 wherein the top plate is manufactured from a compound selected from the group consisting of acrylic, styrene plastic and polymethylpentene.

5. The top plate of Claim 1, further including a frangible section.

6. A disposable assay cassette for use in flow-through assaying, comprising:

a top plate having a first and second surface;

a substantially open channel projecting from the first surface of the top plate to the second surface of the top plate ; and

a membrane affixed to the top plate, wherein the membrane has a binding member for an analyte of interest immobilized thereon.

7. The disposable cassette of Claim 6, further comprising:

a bottom plate having a first and second surface; and

a second channel projecting from the first surface of the bottom plate to the second surface of the bottom plate.

8. The disposable cassette of Claim 6 further comprising a wicking member connected to the membrane.

9. The disposable cassette of Claim 6 wherein the top plate has a plurality of substantially open channels projecting from the first surface of the top plate to the second surface of the top plate.

10. The disposable cassette of Claim 7 wherein the bottom plate has a second plurality of substantially open channels projecting from the first surface of the bottom plate to the second surface of the bottom plate.

11. The disposable cassette of Claim 6 further comprising channel designations affixed to the membrane.

12. The disposable cassette of Claim 6 wherein the membrane is a material selected from the group consisting of nitrocellulose, nylon, polyvinyldifluoridene.

13. The disposable cassette of Claim 6 wherein the membrane is a covalent coupling membrane.

14. An assay apparatus having disposable parts for use in flow-through assaying, comprising:

a removable cassette having:

a top plate having a first and second surface;

a substantially open channel projecting from the first surface of the top plate to the second surface of the top plate ; and

a membrane affixed to the top plate, wherein the membrane has a binding member for an analyte of interest immobilized thereon; and

a base plate receiving the removable cassette, the base plate including a cavity having a vacuum port that is operatively connected to a vacuum source.

15. The assay apparatus of Claim 14 wherein the top plate of the removable cassette has a flange skirting a portion of the top plate that contains the substantially open channel.

16. The assay apparatus of Claim 15 wherein the base plate has a grove that receives the flange skirting a portion of the top plate.

17. The removable cassette of Claim 14, further comprising:

a bottom plate having a first and second surface; and

a second channel projecting from the first surface of the bottom plate to the second surface of the bottom plate.

18. The removable cassette of Claim 14 further comprising a wicking member connected to the membrane.

19. The assay apparatus of Claim 14, further comprising a cover plate having an aperture allowing the exposure of the substantially open channel.

20. A assay kit for use in flow-through assaying, comprising:

a top plate having a first and second surface and a substantially open channel projecting from the first surface of the top plate to the second surface of the top plate;

a membrane having a binding member for an analyte of interest affixed to the top plate; and

a package enclosing the top plate and membrane.

21. A rapid flow-through assaying method, comprising the steps of:

(a) introducing a test sample to a surface of a membrane under vacuum, wherein the membrane has a binding member for an analyte of interest immobilized thereon, and wherein the membrane is connected to a plate having first and second surfaces and a substantially open channel projecting from the first surface to the second surface;

(b) flushing the membrane in order to remove portions of the test sample that are not bound to the membrane; and

(c) reading the results presented on the membrane.

22. An assay cassette, comprising

a top plate having a channel extending therethrough,

a bottom plate for joining to said top plate, and

an engagement mechanism for joining said top plate with said bottom plate and for allowing said top plate to move toward a membrane seated between said top plate and said bottom plate to cause said channel to press against said membrane and to form on the filter a substantially fluid-tight seal around a portion of said filter enclosed by said channel.

23. An assay cassette according to claim 22, further comprising a vacuum port.

24. An assay cassette according to claim 22, wherein said bottom plate includes a vacuum port for applying a negative pressure to one side of a filter seated between said top plate and said bottom plate.

25. An assay cassette according to claim 24, wherein said vacuum port comprises a channel extending through said bottom plate.

26. An assay cassette according to claim 22, wherein at least one of said top plate and said bottom plate includes a frangible section for allowing the respective plate to be divided.

27. An assay cassette according to claim 22, wherein said top plate includes a plurality of channels for forming a plurality of substantially fluid-tight seals on the filter maintained between the top plate and the bottom plate.

28. A device for opening an assay cassette, comprising

a base plate, and

a mechanism for generating a shear force and for applying the shear force to said assay cassette.

29. A device according to claim 28, wherein said mechanism includes a top plate hingedly

mounted to said base plate and being spaced away from said base plate for defining a gap capable of receiving on end of the assay cassette, and wherein pivoting said top plate relative to said base plate provides a shearing force capable of opening said assay cassette.

30. A method of manufacturing an assay cassette, comprising

providing a top plate and a bottom plate capable of being fitted together to define an interior chamber suitable for receiving a filter membrane, and

forming on said at least one of said top plate and bottom plate a frangible section for allowing said respective plate to be divided into two or more components.

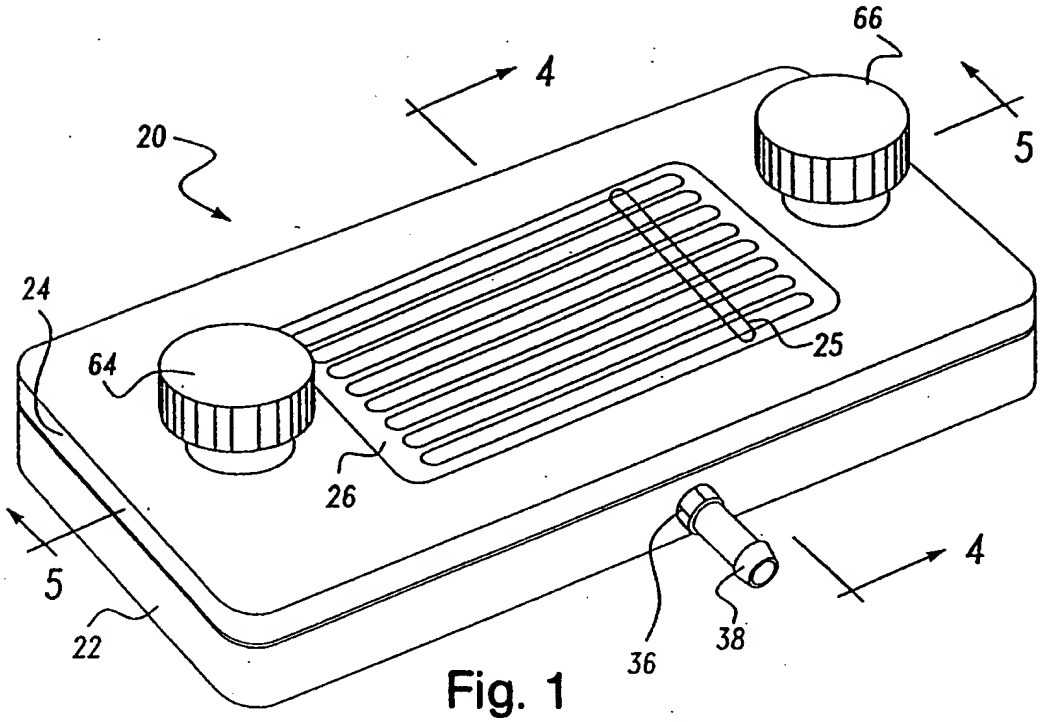

Fig. 1

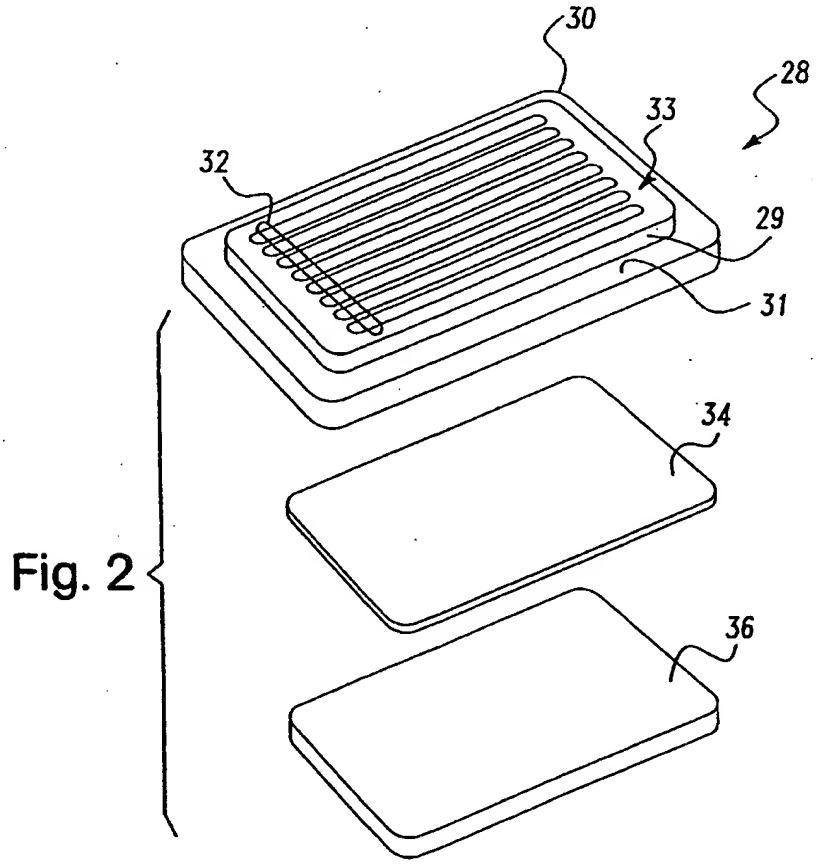

Fig. 2

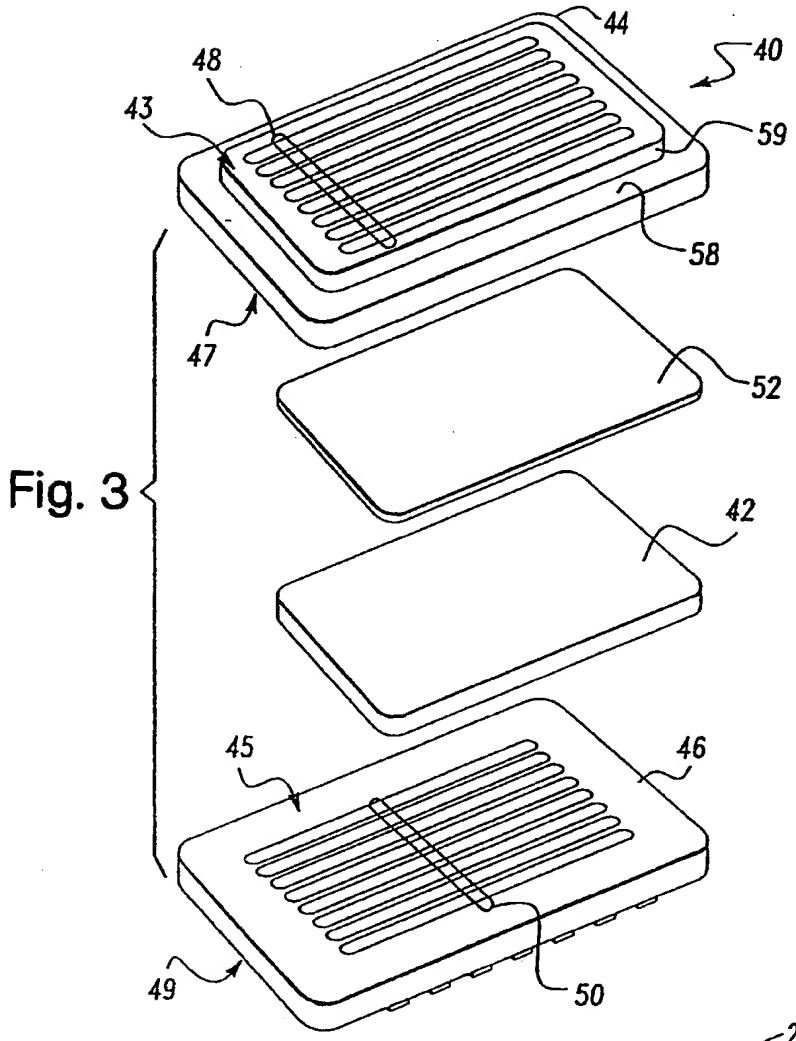
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Fig. 3

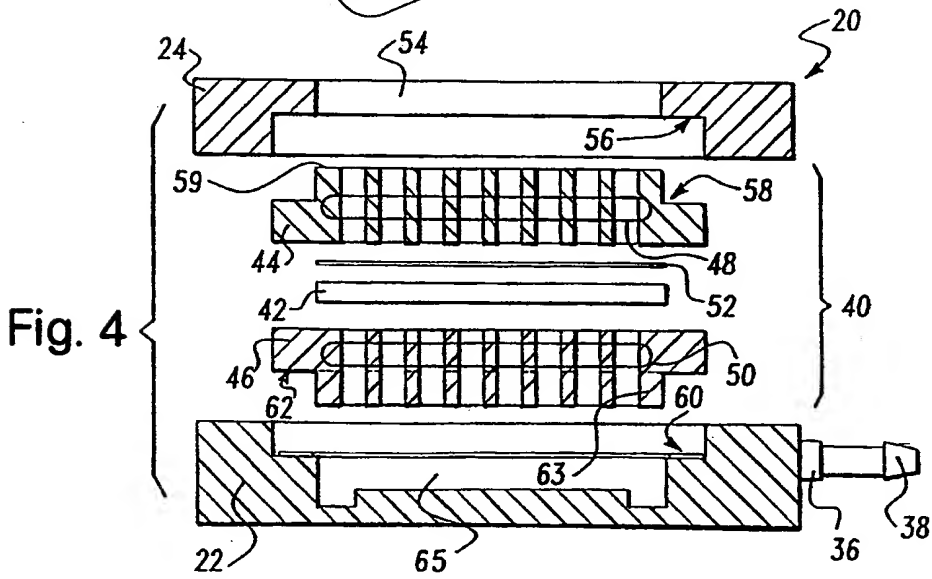
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Fig. 4

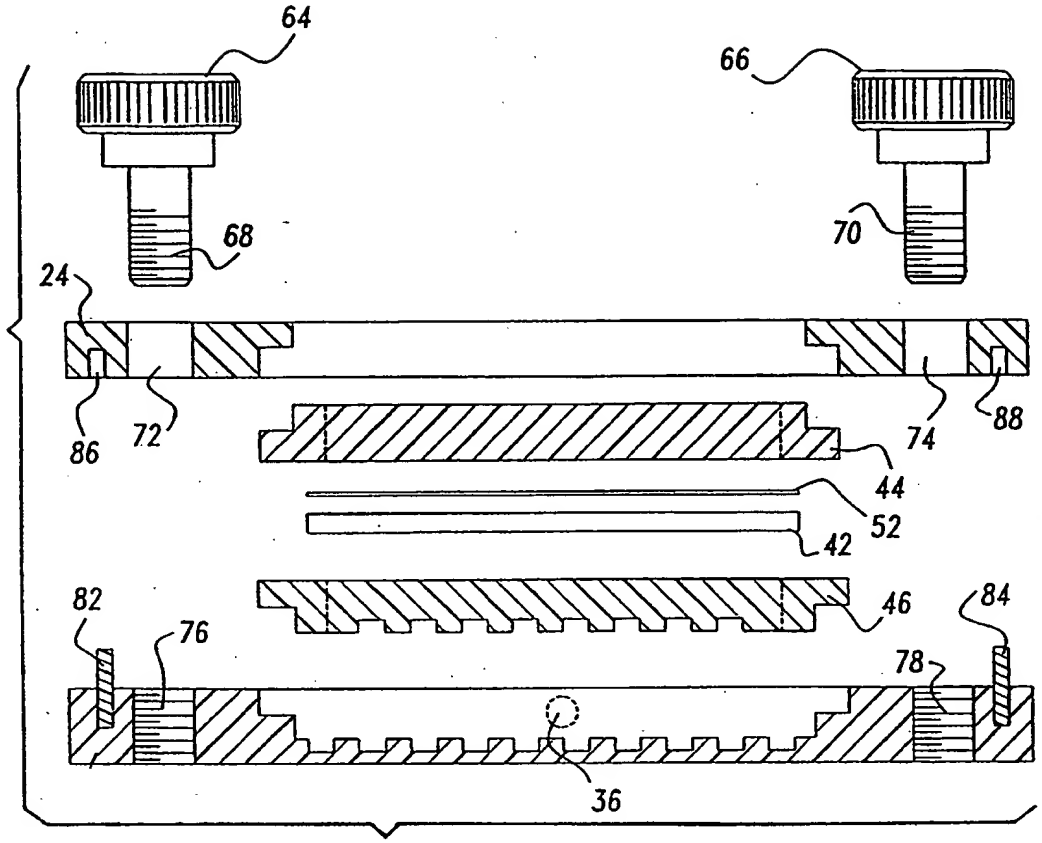
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Fig. 5

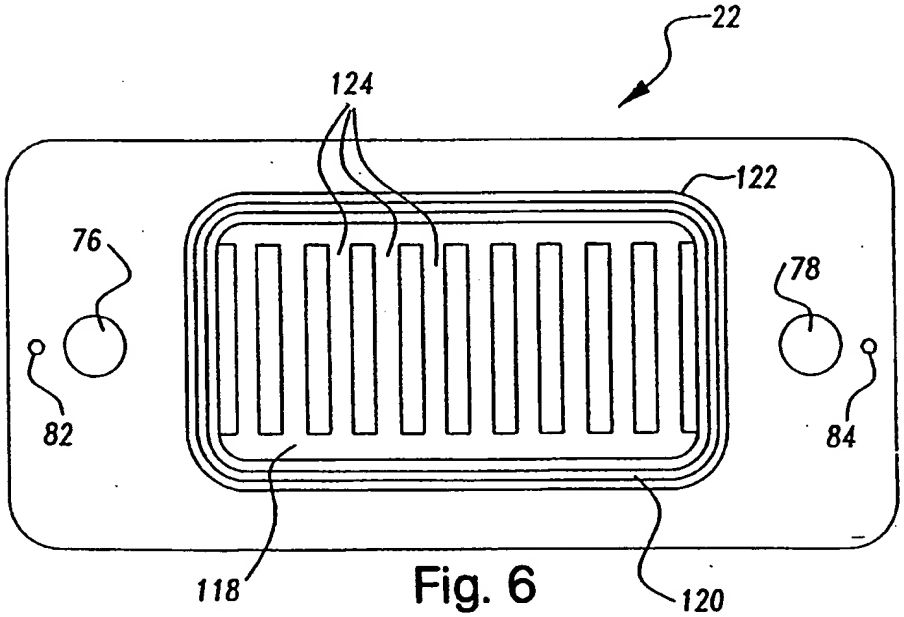
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Fig. 6

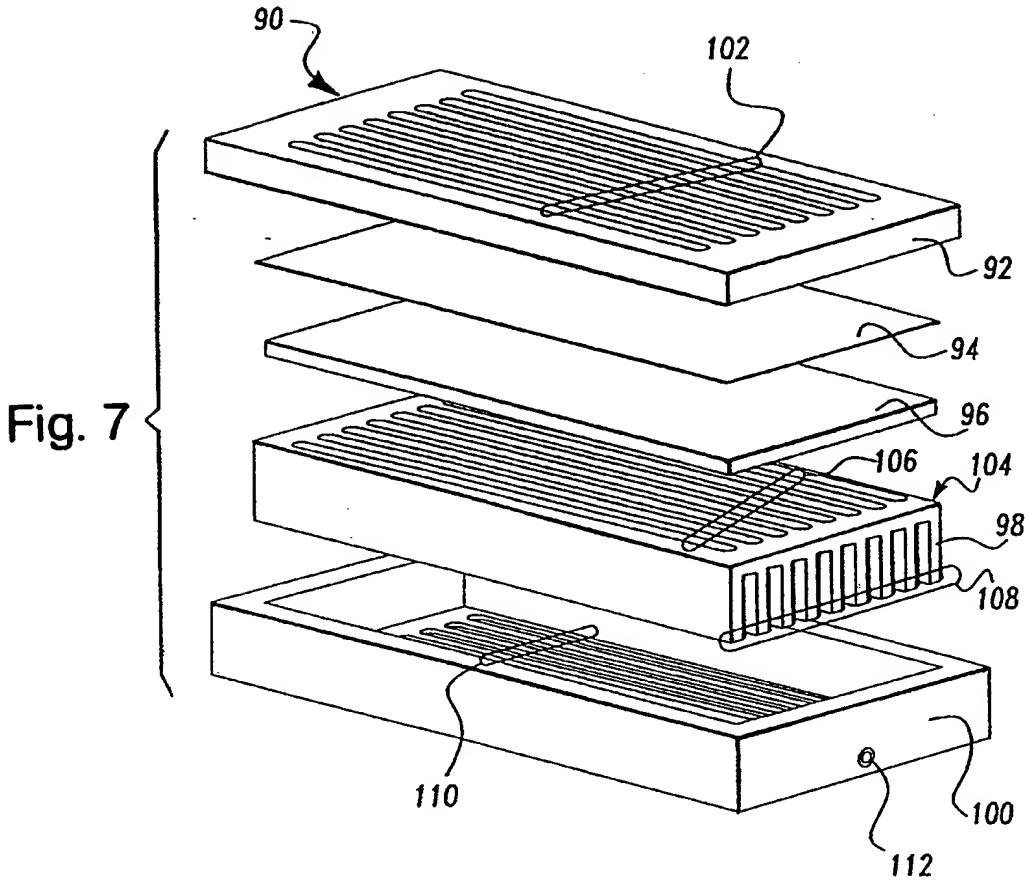
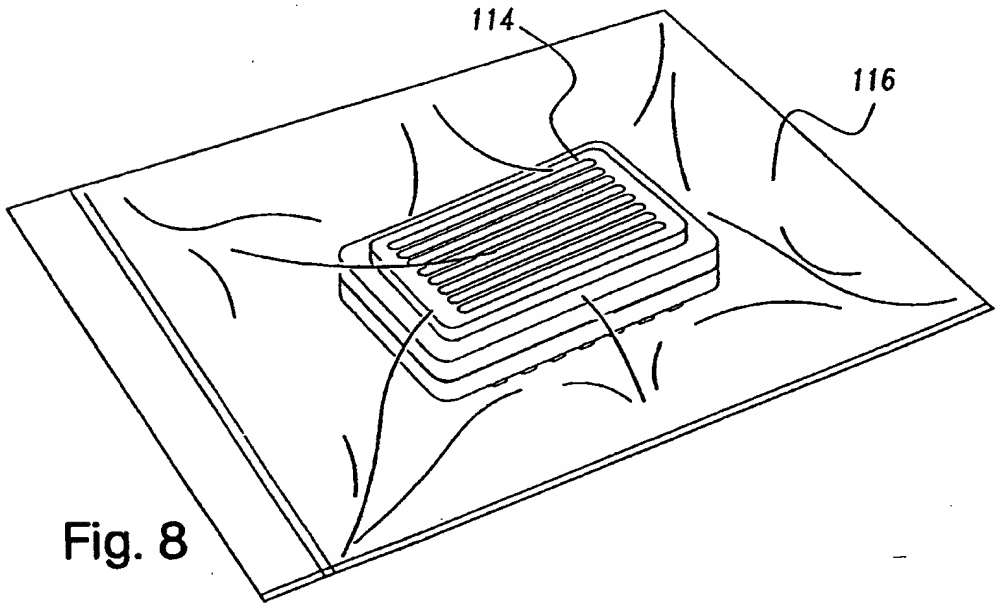


Fig. 7



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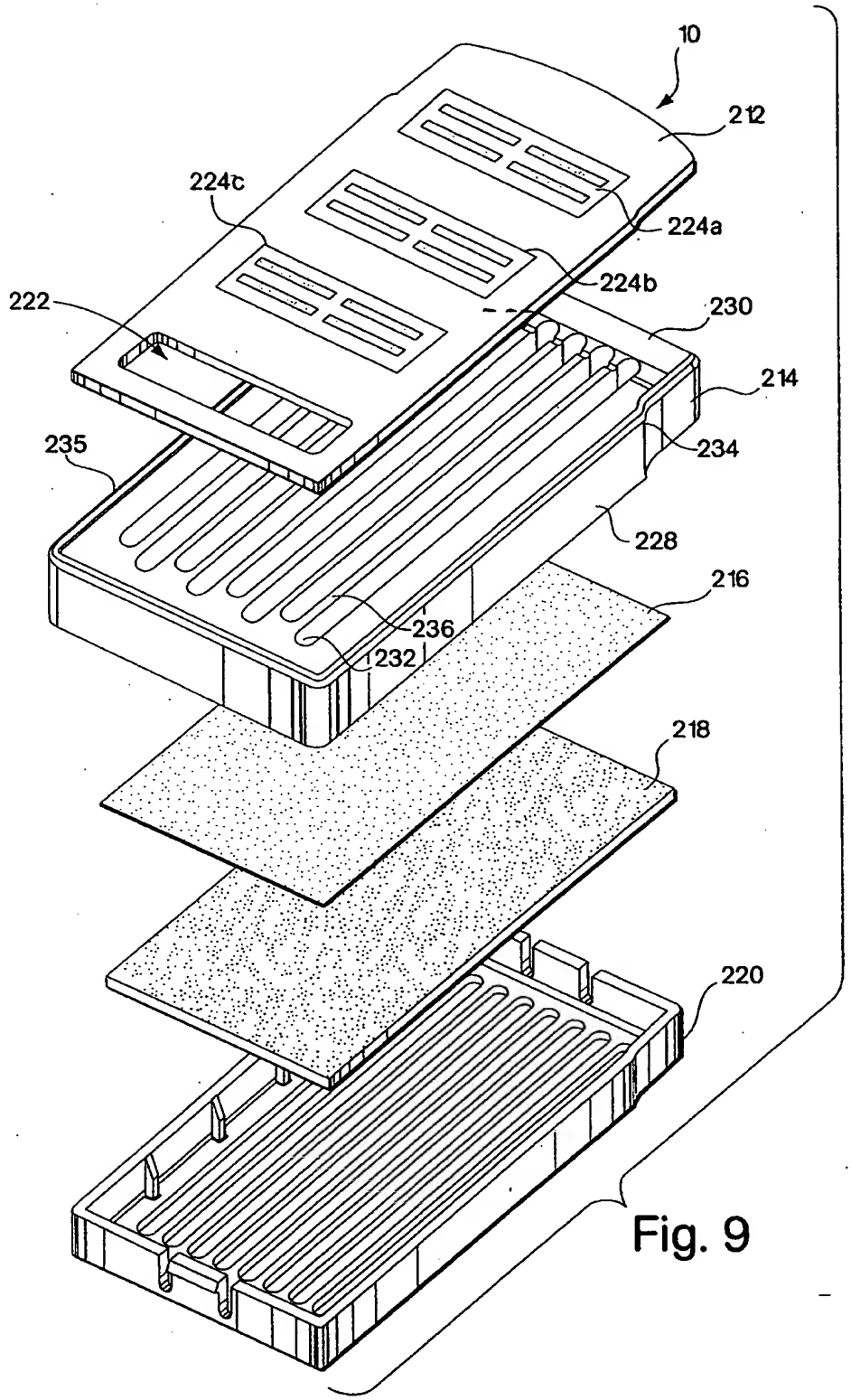
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Fig. 9

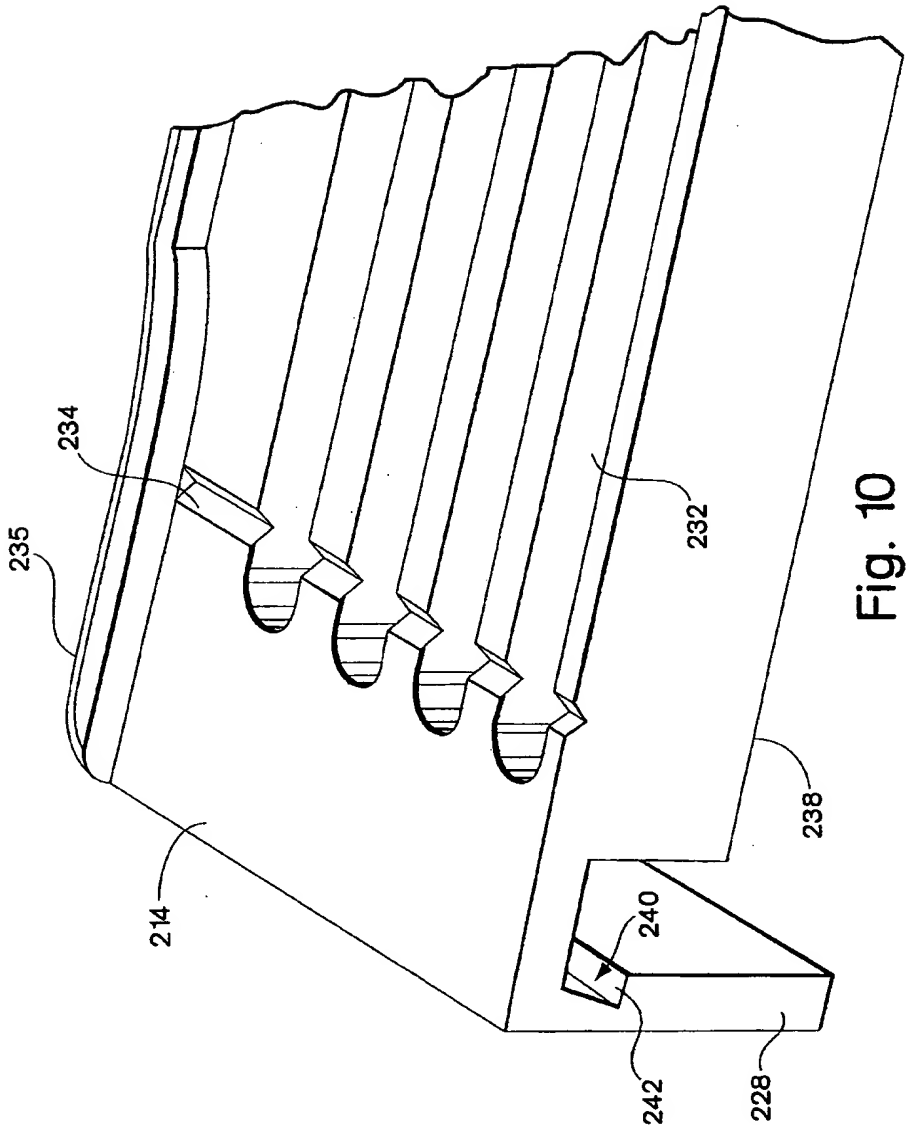

Fig. 10

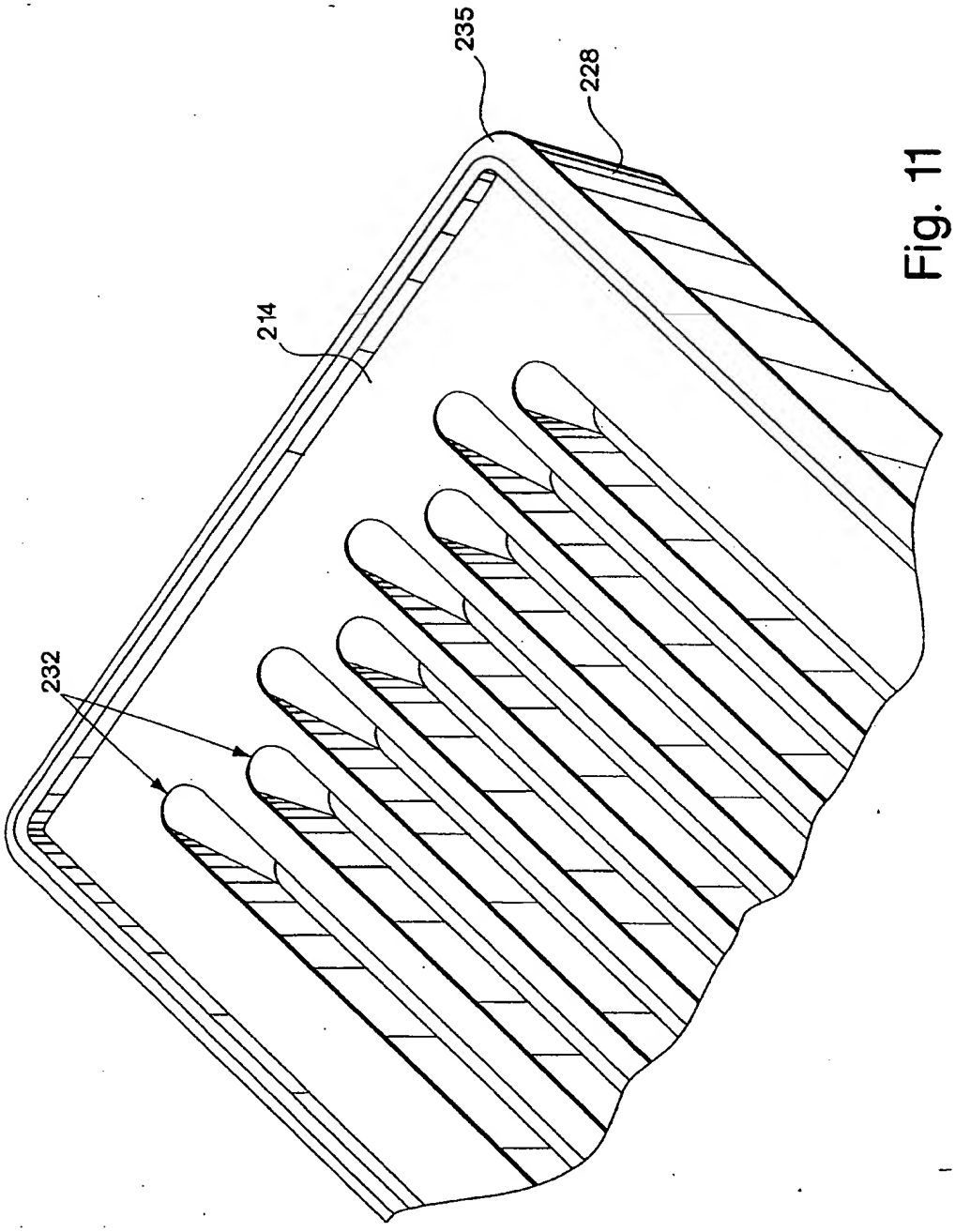

Fig. 11

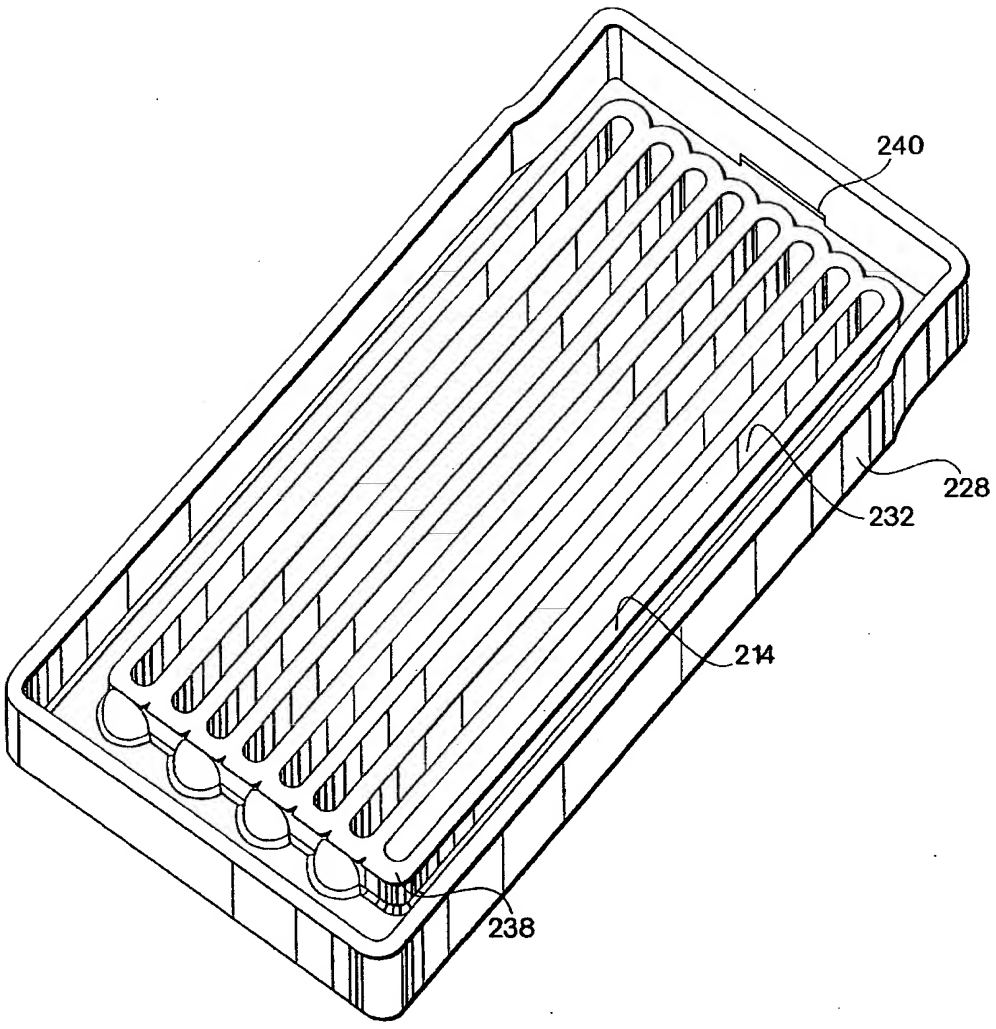

Fig. 12

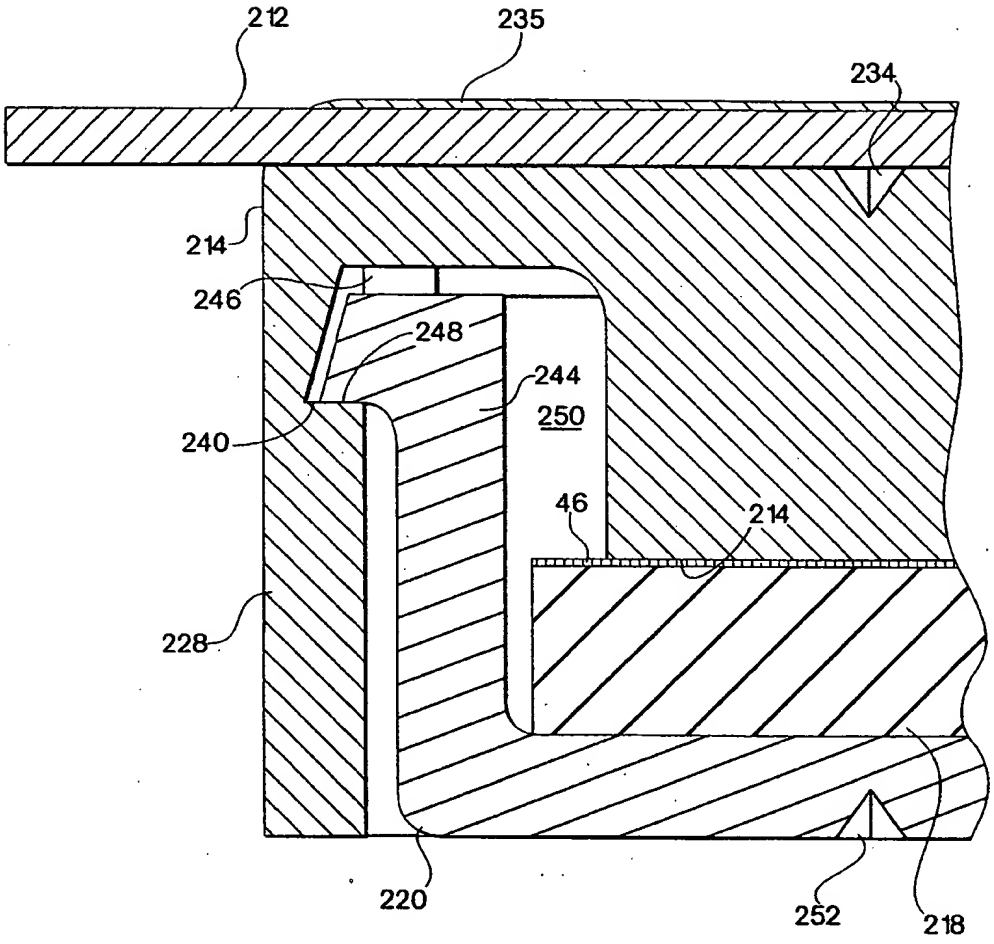

Fig. 13

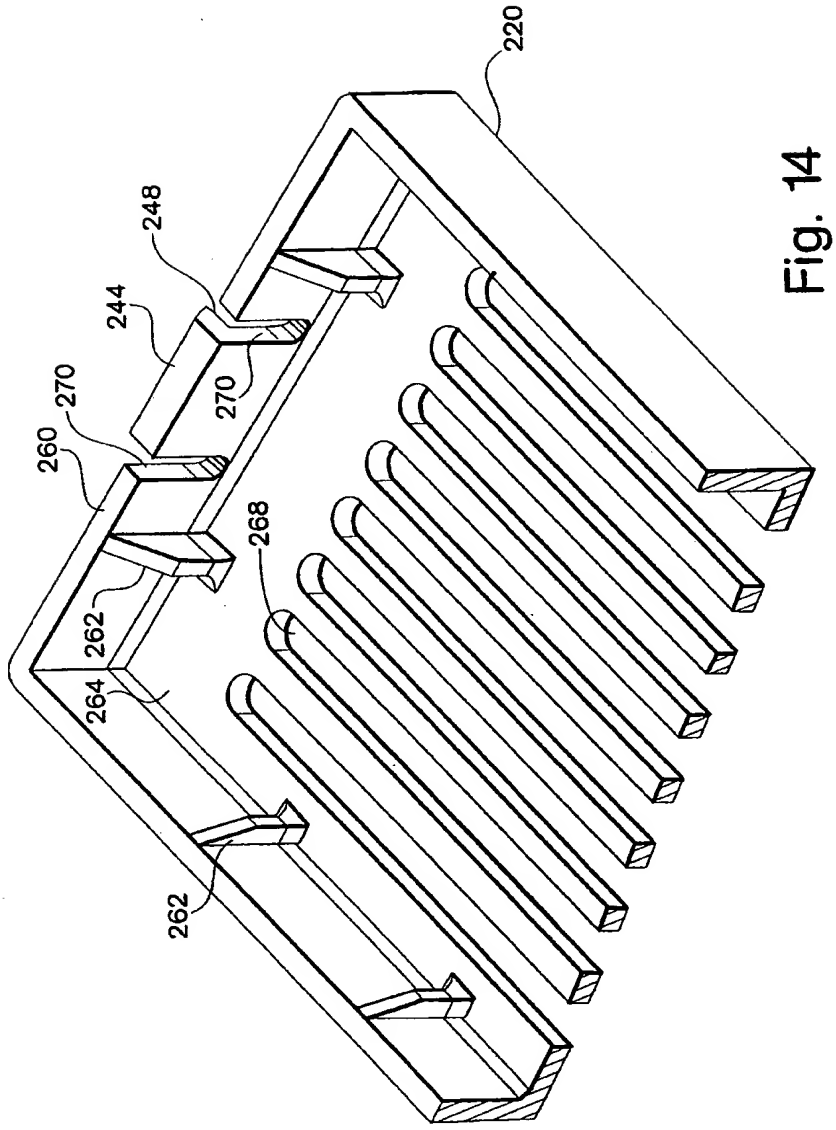

Fig. 14

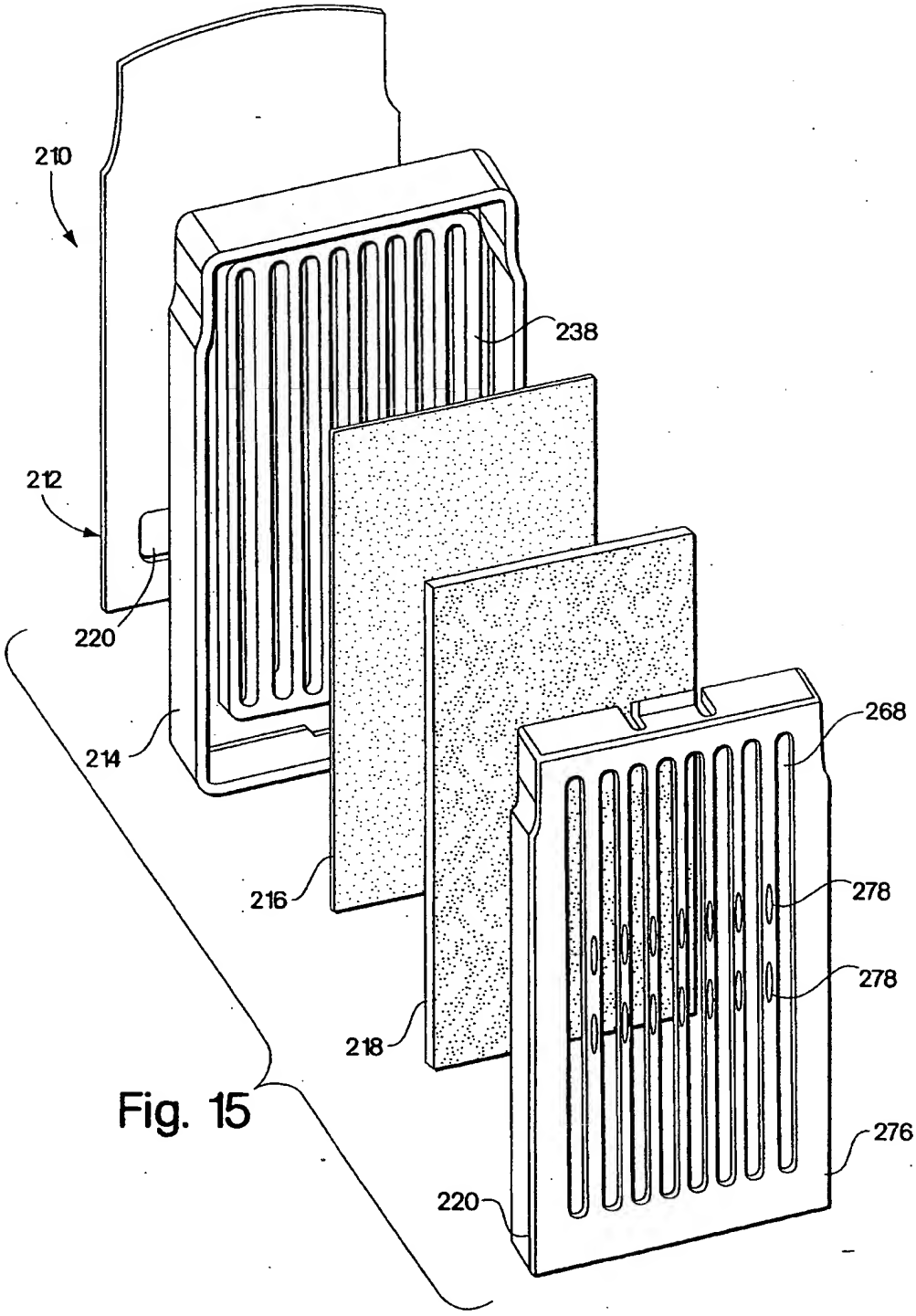

Fig. 15

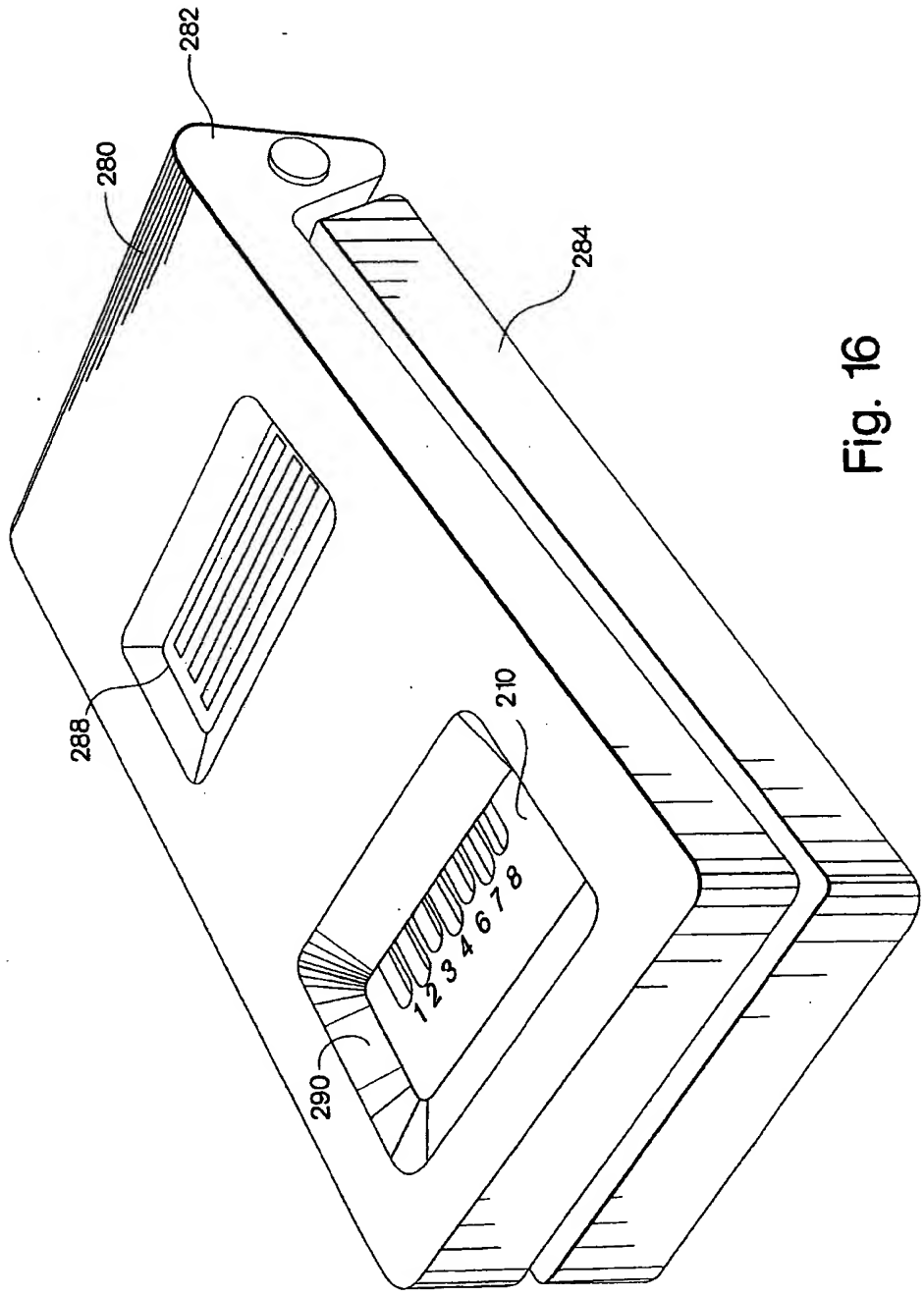

Fig. 16

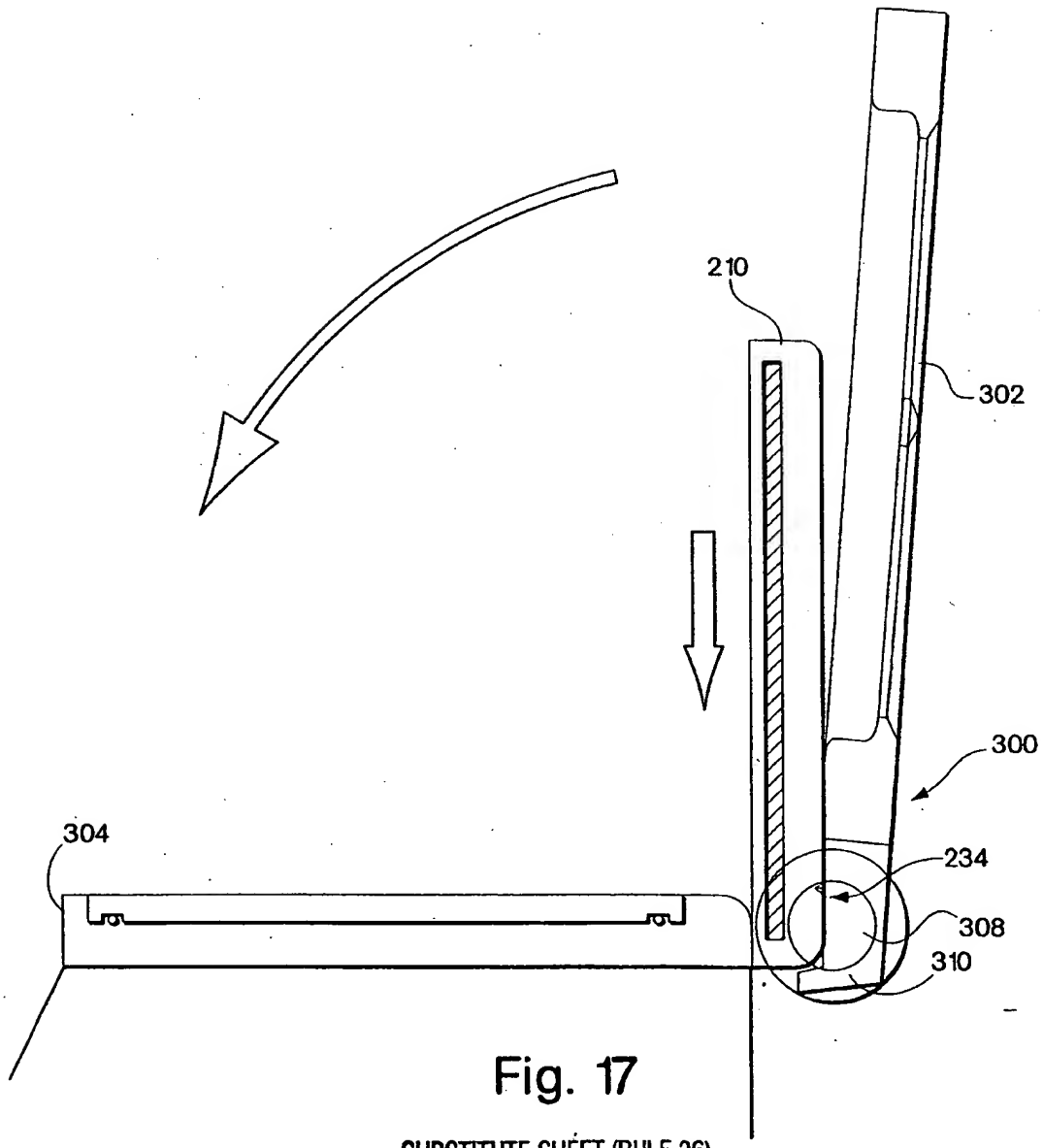

Fig. 17

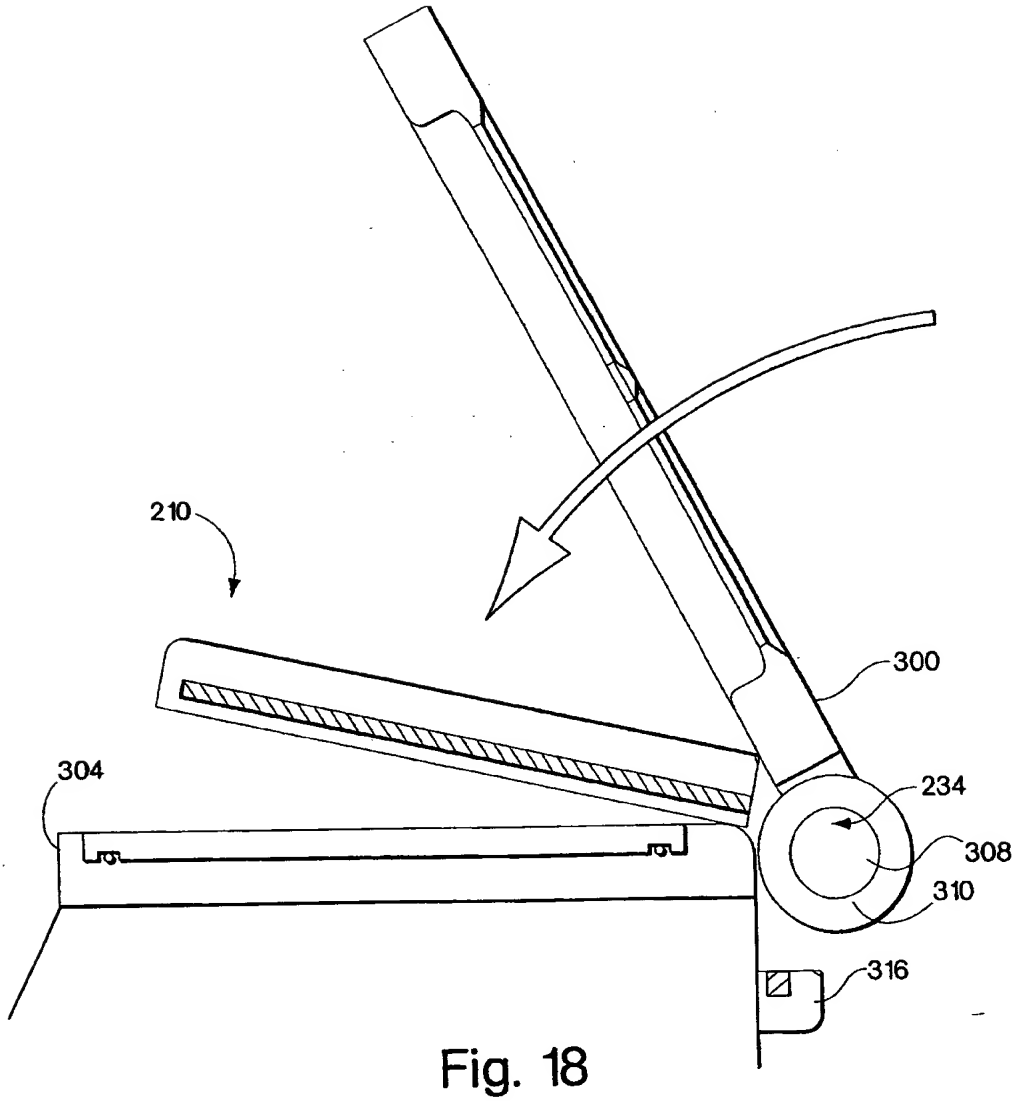

Fig. 18

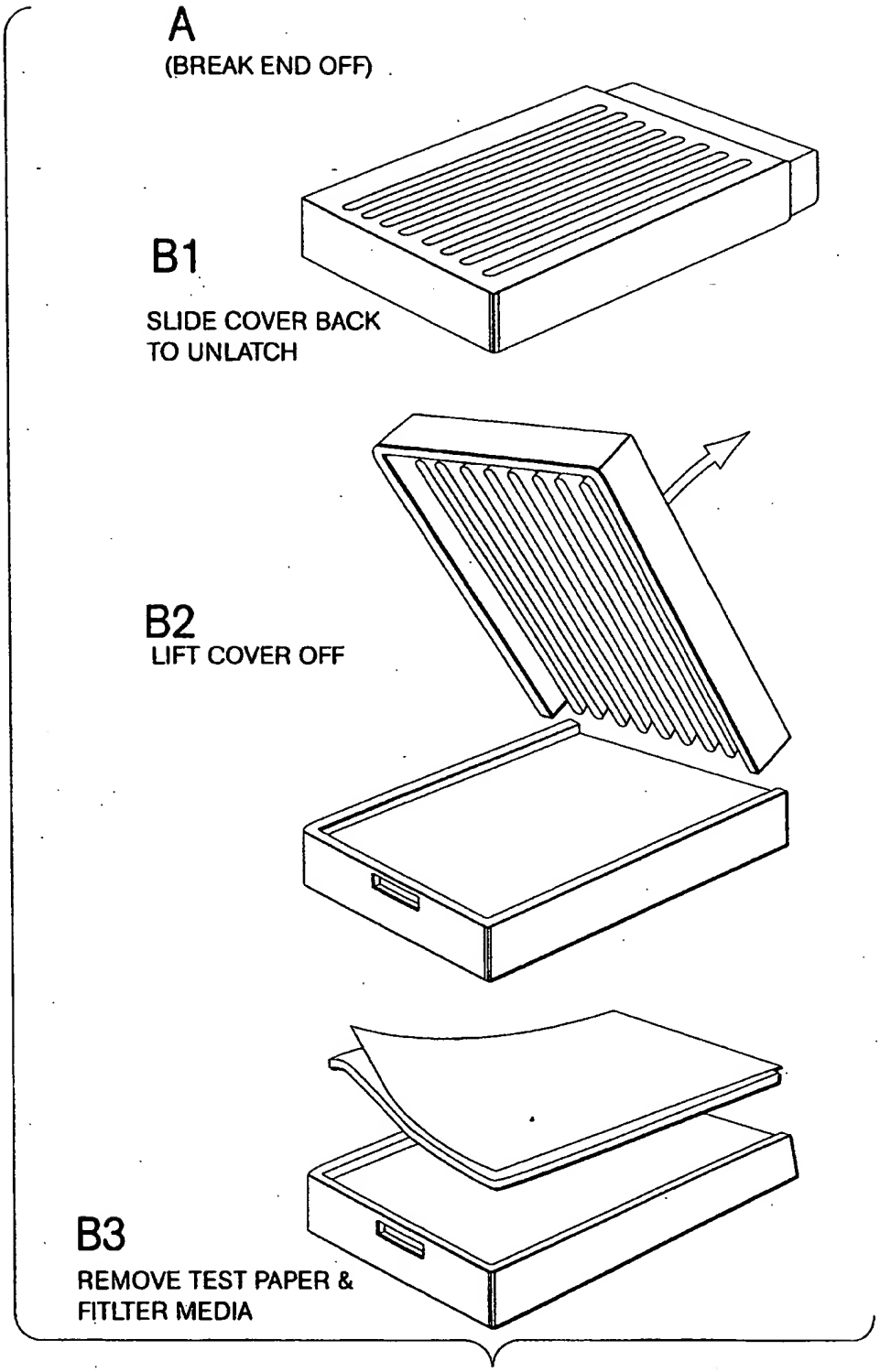
A
(BREAK END OFF)
B1
SLIDE COVER BACK
TO UNLATCH
B2
LIFT COVER OFF
B3
REMOVE TEST PAPER &
FITLTER MEDIA

Fig. 19